Welcome Message

Dear friends and colleagues,

It is our great pleasure in cordially welcoming you to the 8th World Congress on Neurohypophysial Hormones (WCNH2009) to be held in Kitakyushu International Conference Center, Kitakyushu, Japan on the 4th - 8th of September 2009. It is also our pleasure that the 36th annual meeting of the Japan Neuroendocrine Society (JNS) chaired by Professor Yutaka Oiso (Nagoya University Graduate School of Medicine, Nagoya, Japan) will join the WCNH2009 on the 4th - 5th of September at the same place.

The first ever WCNH was held in Japan in 1995. Eight years later the 5th WCNH was held in Kyoto. It is our pleasure to welcome you back to Japan to host the 8th WCNH here in Kitakyushu. We have put together an exciting program that will span all aspects of vasopressin and oxytocin research but will also include recent advances with respect to the post-genomic era. Since the maturation of the human genome project, it has become increasingly important to study not only the molecular mechanisms but also applied aspects such as proteomics and translational research from the bench to the patient's bedside. Neurohypophysial hormones have a close relationship between the brain and peripheral system. They impact on all aspects of our physiology from water homeostasis, the cardiovascular system and reproduction right through to behavior.

We are sure that this meeting will provide a fantastic opportunity to promote our collaborative research, encourage young researchers in the field and strengthen our friendship. In addition, you will be able to enjoy the unique Japanese culture, food, beautiful landscapes and historic sites. We hope that you have an exciting and stimulating experience in the congress and we are very much looking forward to seeing you in Kitakyushu in September 2009.

Sincerely yours,
Yoichi Ueta

University of Occupational
and Environmental Health,
Kitakyushu, Japan

Contents

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Acknowledgments

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Congress Information

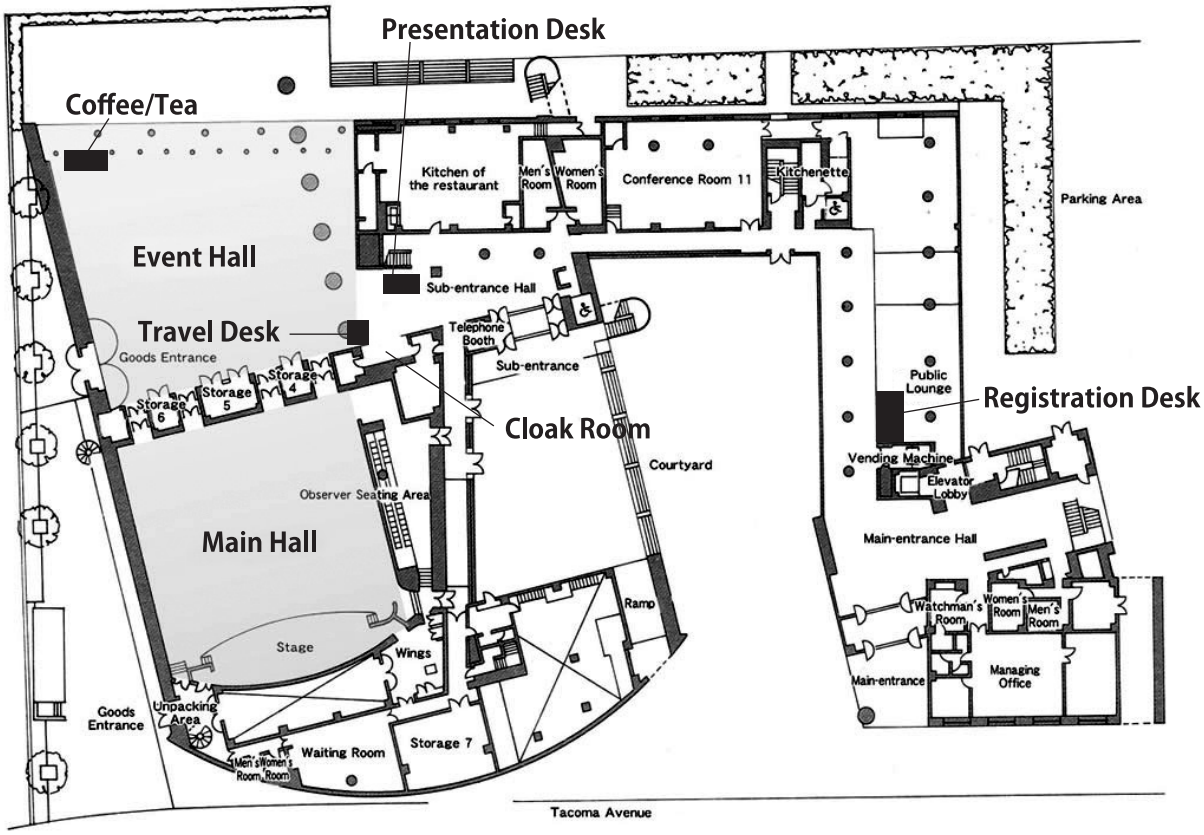
Conference venue: Kitakyushu International Conference Center
3-8-1 Asano, Kokurakita-ku, Kitakyushu 802-0001, Japan
Phone：093-541-5931 Fax：093-541-5928
(http://www.convention-a.jp/eng/cpg/con07.html)

Name badges: Please wear your name badge at all times in the congress building as well as the welcome party and the congress dinner. Regular participants have white badges, speakers' badges show an orange bar, chairs' badges show a blue bar, and those of the organizers and helpers are marked in red.

Registration desk: Registration desk is located 1st floor of the Kitakyushu International Conference Center, and opens on Friday 4th, 14:00h, and remains so throughout the meeting. Services offered include delegate congress registration and any fee payments.

Travel desk: Travel desk is in front of the Main Hall. Services offered include details of the free Sunday afternoon as well as for the social program, personal travel, hotel information and local information.

1st Floor Layout



Onsite registration hours:

Friday 4th	14:00-20:00
Saturday 5th	08:00-17:00
Sunday 6th	08:00-13:00
Monday 7th	08:00-17:00
Tuesday 8th	08:00-11:00

Onsite registration fees:

Regular,WCNH Only	JPY 30,000
Regular,WCNH + JNS Meeting	JPY 32,000
Student,WCNH Only	JPY 15,000
Student,WCNH + JNS Meeting	JPY 16,000
Congress Dinner	JPY 5,000

Speaker presentation: Please bring your PC (Windows/Macintosh) or present your memory stick/CD at the Presentation desk at least one session ahead of the designated symposium or the evening before (for speakers of the morning sessions). There will be an opportunity to preview your presentation before hand.

Poster presentation: All posters can be mounted on Saturday 5th morning (8:00-) and should be removed by completion of the scientific program on Tuesday 8th. Clearly labeled boards can be found in the Event Hall on the 1st floor. Adhesives (pin) will be provided by the congress. There will be two poster sessions, the first on the Saturday 5th (14:00-16:30) for odd-numbered posters and the second on the Monday 7th (13:55-16:15) for even-numbered posters. The poster authors should be attended during the viewing sessions.

Catering/ Lunch: In the coffee breaks coffee, tea and complimentary snacks are offered. Lunch is included registration fee and is provided in front of Main Hall. Cold water is available throughout the sessions.

Internet facilities: PC for e-mail and internet access are available. Please see registration desk for advice.

Welcome party: Buffet style dinner offered to all registrants and accompanying persons at the RIHGA Royal Hotel Kokura (29F) on Friday 4th after the Plenary Lecture 1.

Congress dinner: Congress dinner will take place at the RIHGA Royal Hotel Kokura (4F) on Monday 7th, beginning at 18:15 after the Armin Ermisch Award (18:00). Please bring your name badge.

Sunday afternoon (Social program): Two social programs are offered (a small fee may be required).

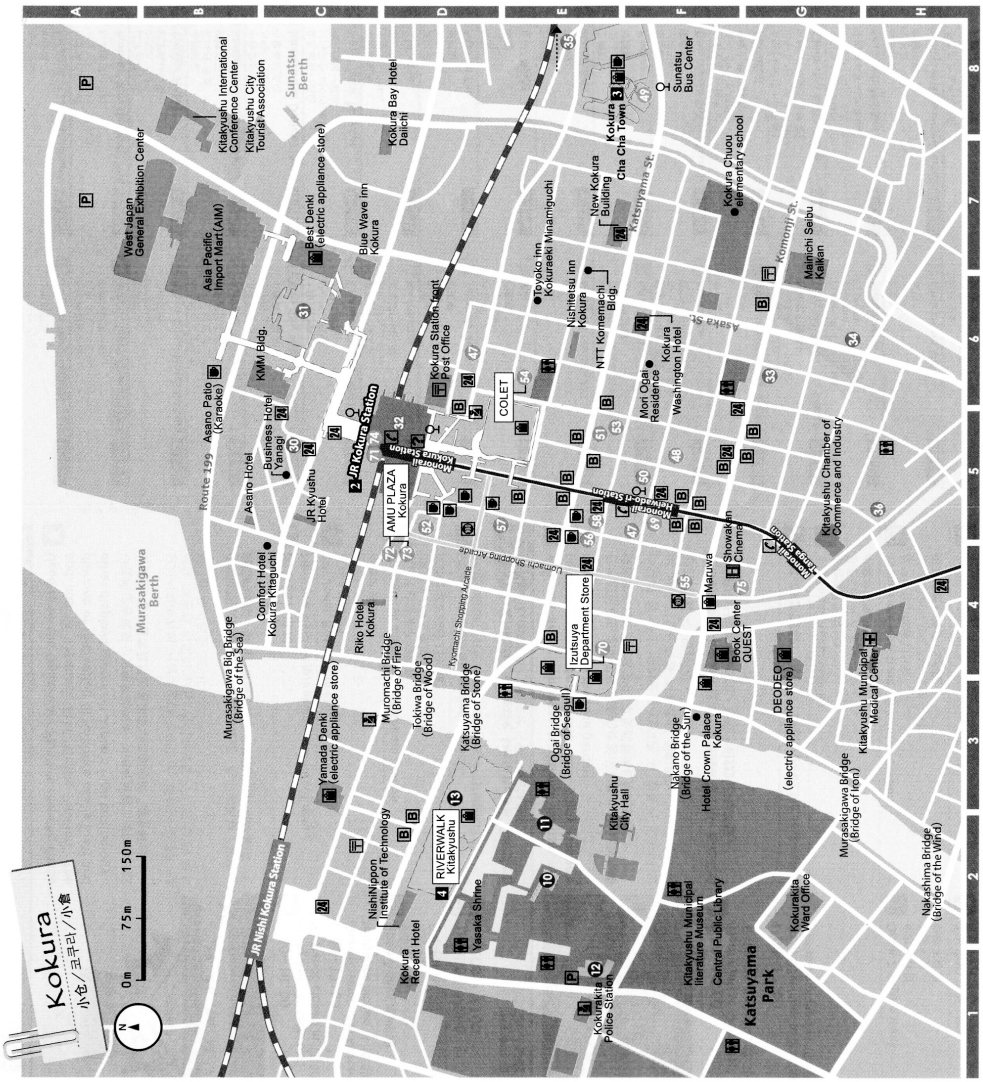
A) Kokura castle & Japanese style tea ceremony

B) Kyushu National Museum & Dazaifu Tenmangu Shrine

Please ask at the travel desk for futher information and book your preferred tour till September 5th 17:00.

Accompanying persons: Need to identify themselves at the registration desk and to register for social program. Please ask a personal travel in the travel desk.

Restaurant & Town map
Information on Kokura



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小倉城

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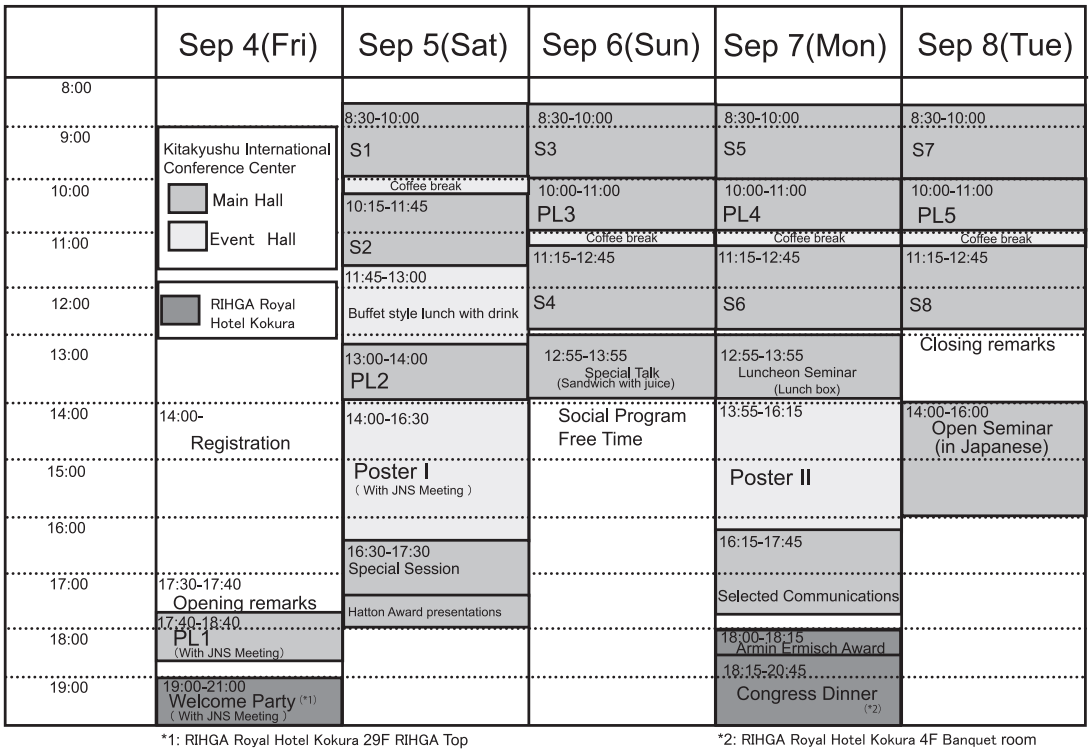
요시카츠
吉勝

Program Overview

Program Overview

WCNH2009 in Kitakyushu, Japan



*1: RIHGA Royal Hotel Kokura 29F RIHGA Top

*2: RIHGA Royal Hotel Kokura 4F Banquet room

SCIENTIFIC PROGRAM

Friday, Sep 4th

14:00- Registration

17:30-17:40 Opening remarks

17:40-18:40 Plenary lecture 1

PL1 John F Morris (Oxford, UK)

Folliculo-stellate cells: sophisticated local controllers in the anterior pituitary

Chair and Introduction: Mitsuhiro Kawata (Kyoto, Japan)

19:00-21:00 Welcome Party

Saturday, Sep 5th

08:30-10:00 Symposium1 Autonomic/Stress

Chair and Introduction: Greti Aguilera (NIH Bethesda, USA)

S1-1 Timothy Bartness (Georgia State University, USA)

Sympathetic and sensory innervation of adipose tissues.

S1-2 Juraj Culman (University Hospital of Schleswig-Holstein, Germany)

Neurokinins in the brain and neuroendocrine regulations under stress

S1-3 Keiichi Itoi (Tohoku, Japan)

The brainstem noradrenergic system in the regulation of neuroendocrine and emotional responses

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10:00 -10:15 Coffee break

10:15-11:45 Symposium2 Forebrain

Chair and Introduction: Tatsushi Onaka (Jichi, Japan)
Geert de Vries (Massachusetts,USA)

S2-1 Andries Kalsbeek (Amsterdam, Nederland)

Vasopressin and the output of the biological clock

S2-2 Robert J. Handa (Loyola Chicago, USA)

Forebrain oxytocin neurons and the integration of estrogen and stress-related signals.

S2-3 Mike Ludwig (Edinburgh, UK)

Vasopressin in the anterior olfactory nucleus; from source to potential functions

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11:45-13:00 Buffet style lunch with drink

13:00-14:00 Plenary lecture 2

PL2 William E Armstrong (Memphis Tennessee, USA)
Properties, performance and plasticity of identified oxytocin and vasopressin neurons in vitro
Chair and Introduction: Celia D Sladek (Colorado, USA)

14:00-16:30 Poster I (With JNS Meeting)

16:30-17:30 Glenn I. Hatton Memorial Symposium (Special Session)
Chair and Introduction: William E Armstrong (Memphis Tennessee, USA)
SS1 William E Armstrong (Memphis Tennessee, USA)
A Scientific Life Fully Realized: Glenn I. Hatton, Ph.D.
SS2 Gareth Leng (Edinburgh, UK)
Glenn Hatton: conjectures and refutations
SS3 Françoise Moos (Bordeaux 2, France)
My tribute to Glenn's findings and concepts on function related plasticity in hypothalamus

Sunday, Sep 6th

08:30-10:00 Symposium 3 Kidney/V2
Chair and Introduction: Eliane Tribollet (McGill, Canada)
Gilles Guillon (Montpellier, France)
S3-1 Gilles Guillon (Montpellier, France)
Functional consequences of vasopressin and corticoliberin receptors co-expression in native and heterologous models
S3-2 Joris Robben (Radboud, The Netherlands)
Intracellular activation of vasopressin V2 receptor mutants in nephrogenic diabetes insipidus by non-peptide sgonists
S3-3 Yumi Noda (Tokyo Medical & Dental University, Japan)
Moving mechanisms of aquaporin-2 water channel

10:00-11:00 Plenary lecture 3
PL3 Mark A Knepper (NIH, USA)
Vasopressin signaling in the renal collecting duct: advances from protein mass spectrometry
Chair and Introduction: Joseph G Verbalis (Georgetown, USA)

11:00 -11:15 Coffee break

11:15-12:45 Symposium4 Clinical / Translational

Chair and Introduction: Takashi Higuchi (Fukui, Japan)
Tadashi Kimura (Osaka. Japan)

S4-1 Bice Chini (CNR Institute of Neuroscience, Italy)
Agonist activation and oxytocin receptor trafficking: role of different G-proteins

S4-2 Regent Laporte(Ferring Research Institute, USA)
Vasopressin V1a receptor agonists in vasodilatory shock: from bedside to bench and back

S4-3 Masamitsu Nakazato (Miyazaki, Japan)
Discovery of novel neuropeptides NERPs that suppress vasopressin secretion

12:55-13:55 Special Talk (Sandwich with juice)

Chair and Introduction: John A Russell (Edinburgh, UK)

ST1 Roger Acher (Paris VI, France)
Structural evolution of neurohypophysial hormones, their precursors and their receptors

ST2 Torsten M Reinheimer (Ferring Pharmaceuticals A/S, Denmark)
Oxytocin's centenary:Discovery, synthesis, drugs, current and future indications

Monday, Sep 7th

08:30-10:00 Symposium5 Central

Chair and Introduction: Jeffrey Tasker (Tulane, USA)

S5-1 Jean Marc Israel (Neurocentre Magendie, France)
Intrinsic versus synaptic control in magnocellular neurones?

S5-2 Colin Brown (Otago, New Zealand)
Dehydration-induced plasticity in autocrine modulation of vasopressin neuron activity

S5-3 Haruhiro Higashida (Kanazawa, Japan)
Stress-induced behavior and plasma oxytocin levels during development are regulated dually by breast milk and hypothalamic ADP-ribosyl cyclase of CD38 in mice

10:00-11:00 Plenary lecture 4

PL4 Robert Schrier (Denver Colorado, USA)
Vasopressin and oxytocin in pregnancy: water channels, ion transporters, and receptor antagonists

Chair and Introduction: San-e Ishikawa (Jichi, Japan)

11:00 -11:15 Coffee break

11:15-12:45 Symposium6 Molecular

Chair and Introduction: W. Scott Young, 3rd (NIH, USA)

S6-1 Lesley Stewart (Bristol, UK)

Dehydration switches control of sympathetic activity from forebrain to hindbrain

S6-2 Søren Rittig (Aarhus university hospital, Nederland)

Translational Aspects of Arginine Vasopressin

S6-3 Hiroshi Arima (Nagoya, Japan)

Polyuria progressed in the absence of vasopressin neuron loss in a mouse model for familial neurohypophysial diabetes insipidus

12:55-13:55 Luncheon Seminar (Lunch box)

Chair and Introduction: Yutaka Oiso (Nagoya, Japan)

LS Joseph G Verbalis (Georgia, USA)

Vasopressin Receptor Antagonists: Past, Present and Future

13:55-16:15 Poster II

16:15-17:45 Selected Communications

Chair and Introduction: Alison J Douglas (Edinburgh, UK)
Ken'ichi Yamaguchi (Niigata, Japan)

18:00-18:15 Armin Ermisch Award

Chair and Introduction: Rainer Landgraf
(Max Planck Institute of Psychiatry, Germany)

18:15-20:45 Congress Dinner

Tuesday, Sep 8th

08:30-10:00 Symposium7 Behavior

Chair and Introduction: Sonoko Ogawa (Tsukuba, Japan)
Katsuhiko Nishimori (Tohoku, Japan)

S7-1 Alison J Douglas (Edinburgh, UK)

Control of oxytocin neuron behavior perinatally: role of neuronal inputs from the uterus

S7-2 Larry Young (Emory, USA)

Oxytocin, vasopressin and affiliative behavior:

genetics, neural circuitry and experience
S7-3 Oliver J. Bosch (Regensburg, Germany)
Brain arginine vasopressin is an important regulator of maternal care and aggression

10:00-11:00 Plenary lecture 5
PL5 Rainer Landgraf
(Max Planck Institute of Psychiatry, Germany)
The vasopressin gene and anxiety- from clue to causality
Chair and Introduction: Yoichi Ueta
(University of Occupational and Environmental Health, Japan)

11:00 -11:15 Coffee break

11:15-12:45 Symposium8 Comparative
Chair and Introduction: Yoshio Takei (Tokyo, Japan)
Roger Acher (Paris Ⅵ, France)
S8-1 Soojin Ryu (Max Planck Institute of Psychiatry, Germany)
Genetic Analysis of Hypothalamic Neuron Development in Zebrafish
S8-2 Jawes L Goodson (Indiana, USA)
Nonapeptides and the Evolution of Social Group Sizes in Finches: Touchstones for All Vertebrates?
S8-3 Masakazu Suzuki (Sizuoka, Japan)
Molecular diversity of aquaporins closely associated with water adaptation strategy in anuran amphibians

13:00- Closing remarks

14:00-16:00 Open Seminar (in Japanese)
Yutaka Oiso (Nagoya, Japan)
Yoshio Takei (Tokyo, Japan)
Chair and Introduction: Yoichi Ueta, Hiroshi Yamashita
(University of Occupational and Environmental Health, Japan)

Plenary and symposia Abstracts

Folliculo-stellate cells: sophisticated local controllers in the anterior pituitary

John Morris, Helen Christian

Department of Physiology, Anatomy & Genetics, University of Oxford, Oxford OX1 3QX, U.K.

Most research on the anterior pituitary has concentrated on its classical endocrine cells, and its control, both by hypothalamic releasing/inhibiting hormones and feedback of systemic hormones. Folliculo-stellate (F-S) cells are usually described as “non-endocrine” and have been paid scant or no attention. However, they constitute a functional gap junction-coupled syncytium throughout the anterior pituitary, produce numerous signals (annexin 1, NO, VEGF, IL-6, follistatin, activin) which influence surrounding cells, and have many receptors which allow them to respond to local and systemic signals. They may also have stem cell capabilities.
We have shown that F-S cells are important local controllers in the stress response. They are responsible for early-delayed (30min-3h) cortisol feedback but can also modulate glucocorticoid levels via their 11β-ODSD enzymes. Cortisol increases transcription of annexin 1 but also its externalization from the cells (via an ABC-transporter system). The externalized annexin 1 inhibits the secretion of most classic anterior pituitary hormones; it occurs at specific points on the F-S cell membrane where they attach to endocrine cells to form a sort of ‘paracrine synapse’. The stress response is markedly sexual dimorphic; F-S cells have receptors for both estrogen and testosterone, and knockout of either annexin 1 or its putative receptor causes a large increase in ACTH cells in male but not female offspring. ATP, which is released when secretory granules from endocrine cells are exocytosed, also causes externalization of annexin 1 from F-S cells creating an ultrashort negative feedback loop. ATP also causes a transient increase in intracellular calcium, a signal by which F-S cells are linked via gap junctions. In this way the effects of secretion in one part of the gland can be communicated throughout the gland. We also show that the secretion of VEGF by F-S cells is controlled via K-ATP channels. This is important in view of the presumed low oxygen tension in a gland which derives its blood supply from portal veins, and may also be relevant to the vascularisation of pituitary tumours.

S1-1

Sympathetic and Sensory Innervation of Adipose Tissues.

Timothy J. Bartness, Yogendra Shrestha, Cheryl H. Vaughan and C. Kay Song

Department of Biology, Georgia State University and Center for Behavioral Neuroscience, Atlanta, GA 30302-4010, USA

Despite the historical emphasis on the control of white adipose tissue (WAT) metabolism via circulating factors (e.g., epinephrine [EPI]; insulin) and the communication from WAT to brain by secreted factors such as leptin, there is a growing appreciation of the control of WAT by its sympathetic nervous system (SNS) innervation and of its sensory innervation as a neural conduit to the brain. A primary reason for this neural focus is the ability to trace efferent and afferent circuits to and from WAT, respectively, using transneuronal viral tract tracers, as we first did for WAT SNS innervation using pseudorabies virus (PRV) and for WAT sensory innervation using the H129 strain of herpes simplex 1 virus. We also have identified some of the neurochemicals involved in these circuits including the melanocortin 4-receptor (MC4-R) found on ~60% of brain neurons comprising the SNS outflow to WAT across the neuroaxis and leptin receptors (Ob-Rb) found on dorsal root ganglia neurons that are part of the sensory inputs to the brain. Functionally, central MC4-R agonism increases the SNS drive to WAT (norepinephrine turnover [NETO]), albeit differentially across the WAT pads. Moreover, we recently adapted in vitro Western blot measures of the phosphorylation of perilipin A and hormone sensitive lipase (pPerilipin A, pHSL), biochemical processes necessary for catecholamine-stimulated lipolysis, as in vivo markers of SNS-stimulated lipolysis on a fat pad-specific basis. Only WAT depots showing increased NETO after central MC4-R agonism had increased pPerilipin A and pHSL. Glucoprivation also markedly increases WAT NETO and is associated with increases in sensory nerve electrophysiological activity suggesting monitoring of lipolysis. Indeed, preliminary studies indicate single neurons in the brain that receive sensory inputs from WAT and are also part of the SNS outflow to WAT, as revealed by injections of the SNS tracer (PRV) and the sensory tracer (H129) both into the same WAT pads -- suggesting a lipolytic neural feedback loop. The presence of Ob-Rbs on some of these neurons suggests an additional role of leptin as an part of this neural feedback loop, rather than serving only as a humoral signal. Collectively, these data exemplify the importance of the SNS and sensory innervation of WAT for control of lipid metabolism.

Neurokinins in the brain and neuroendocrine regulations under stress

Juraj Culman and Yi Zhao

Institute of Experimental and Clinical Pharmacology, University Hospital of Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany

Substance P (SP) and neurokinin A (NKA), which belong to the tachykinin family of peptides, interact predominantly with the NK_1 and NK_2 receptor, respectively. Both peptides induce in the brain an integrated cardiovascular, endocrine and behavioural response closely related to the defence reaction. NK_2 receptors are only sparsely expressed in the adult brain. The cardiovascular and behavioural responses to SP and NKA are identical indicating that NKA in the brain may exert its effects through activation of NK_1 receptors. Experiments carried out in mice lacking the NK_1 receptor (NK1-KO mice) and employing the high-affinity, non-peptide NK_1 and NK_2 receptor antagonists in wild-type mice revealed that an interaction between the NK_1 and NK_2 receptor mediates the central effects of NKA. SP and serotonin (5-HT), which is a potent activator of the hypothalamo-pituitary-adrenocortical axis (HPA) in the brain, have been implicated in the pathophysiology of stress-related disorders, such as anxiety and depression. NK_1 receptor antagonists were proposed to possess antidepressant activity. In line with this assumption, blockade of central NK_1 and NK_2 receptors attenuates the cardiovascular, endocrine and behavioural responses to stress and reduces the neuronal activity in the brain areas involved in the generating of these responses, such as the paraventricular nucleus (PVN). Paradoxically, SP in the brain exerts a tonic inhibition on the HPA, as stimulation of forebrain NK_1 receptors inhibits the release of ACTH. SP increases the turnover of 5-HT in the raphe nuclei, where 5-HT cell bodies are localised, but abolishes the release of 5-HT in the PVN. Stress up-regulates the expression of the corticotropin-releasing factor, increases plasma ACTH and accelerates the 5-HT turnover in the raphe nuclei more effectively in NK_1-KO- than in wild-type mice, indicating that SP reduces the activity of the HPA and 5-HT cell bodies. The tonic inhibition of the HPA by SP is not in line with the proposed antidepressant activity of NK_1 receptor antagonists.

S1-3

The brainstem noradrenergic system in the regulation of neuroendocrine and emotional responses

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The locus coeruleus (LC) is the largest noradrenergic (NA) nucleus in the brain which sends out axonal projections to almost all brain regions. The LC has been implicated in sleep/wakefulness, attention and alertness, cognition, or fear and anxiety. Despite considerable evidence suggesting the relationship between the central NA system and fear/anxiety states, previous animal studies have not been able to demonstrate sheer involvement of the LC in mediating fear or anxiety. It is not clear, either, whether the LC also mediates the transmission of stress signals to the hypothalamus whereas the medullary NA nuclei, including the A1 and A2, are involved in the regulation of neuroendocrine stress responses.
We employed two distinctive methods for studying the possible involvement of the LC in emotional and neuroendocrine responses. First, the LC was totally ablated following injection of the immunotoxin [anti-human interleukin-2 receptor (hIL2R) conjugated with pseudomonas exotoxin] into the LC of a transgenic mouse in which the hIL2R is expressed under the promoter of dopamine beta hydroxylase. Second, mice were treated with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), that ablates selectively the axon terminals origination from the LC. Depending on the method of ablation and/or the time following intervention, the parameters in a battery of anxiety-like behaviors shifted to the opposite direction, or they did not change significantly from those in the control. Gradual recovery of the function of the NA system following DSP-4 administration could be related to the apparently equivocal results. Alternatively, the anxiety-like behavior could be related to the balance of the availability of multiple neurotransmitters in the brain.

Vasopressin and the output of the biological clock

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In mammals, the daily rhythms in behavior and physiology are generated and orchestrated from within a biological clock located in the anterior hypothalamus. The location of this clock within the hypothalamic suprachiasmatic nucleus (SCN) was discovered in the early 1970s. More recently, it became clear that the endogenous rhythm of the SCN is generated by a suite of clock genes. Despite this vast increase in recent knowledge, the manner in which individual SCN neurons are assembled to create an integrated tissue clock that can govern the sleep/wake behavior of the whole animal is still unknown. The first SCN transmitter to be demonstrated was vasopressin (VP), although at that time its function as a neurotransmitter was not yet recognized. Due to its pronounced day/night rhythm in CSF, VP was characterized as a humoral output of the SCN, and to date it is still the only SCN output that has been demonstrated to be secreted in a daily rhythm in vivo. Clearly the daily fluctuations of VP in the CSF are a result of the day/night rhythm in the firing rate of VP-containing SCN neurons. Despite its early discovery, the interest in VP as an important clock output rapidly disappeared when no gross abnormalities could be detected in the sleep/wake rhythms of the Brattleboro rat. However, more recent observations renewed the interest in VP as an important clock-controlled output gene. We showed that, in the rat, VP derived from the SCN has a strong inhibitory effect on the release of adrenal corticosterone and is an important component in the generation of a daily rhythm in plasma corticosterone concentrations, as well as the LH-surge in female animals. On the other hand, the vasopressinergic output of the clock does not seem to be involved in the control of the daily rhythms in melatonin release or hepatic glucose production. Further results show that with regard to the daily corticosterone rhythm in diurnal and nocturnal rodents, temporal information is carried along the same pathway from the SCN to its target areas, but the response of the target area may be quite different. We propose that the reversed response to VP is due to a change in the phenotype of the target neurons that are contacted by the SCN efferents, i.e. glutamatergic instead of GABAergic.

S2-2

Forebrain oxytocin neurons and the integration of estrogen and stress-related signals.

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Estrogens have profound effects on the hormonal and behavioral responses to stress. These actions of estradiol are mediated through its interactions with two estrogen receptors (ER), ERalpha and ERbeta. We have recently demonstrated that these two receptors act in opposition to regulate hormonal stress responses and anxiety-related behaviors, in a rodent model. When delivered to ovariectomized female rats, ER beta agonists have anxiolytic actions and inhibit the activity of the hypothalamo-pituitary-adrenal (HPA) axis, whereas ERalpha agonists increase anxiety and HPA activity. ERbeta is found in high levels of oxytocin neurons in the paraventricular nucleus of the hypothalamus and this area provides the bulk of oxytocin to the forebrain. Consequently, we have explored the role of oxytocin (and vasopressin) in mediating the actions of ERbeta on anxiety related behaviors and stress response. Our studies demonstrate that the anxiolytic actions of ERbeta agonists are present when administered to wild-type but not to oxytocin-knockout mice. Correspondingly, the anxiolytic actions of ERbeta agonists can be prevented by treatment with oxytocin antagonists delivered to the 3rd ventricle shortly before behavioral testing. Our studies also show that neuronal activity (as determined by monitoring c-fos expression) is induced by anxiety-like behavior testing in regions in and around the central nucleus of the amygdala and the bed n. of the stria terminalis. The c-fos responses are enhanced by treatment with ERbeta agonists in wild type but not oxytocin knockout or ERbeta knockout mice. Taken together, these data begin to describe the neural circuitry that underlies the diverse actions of estradiol on hormonal and behavioral responses to stress.
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Vasopressin in the anterior olfactory nucleus; from source to potential functions

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The anterior olfactory nucleus (AON) is a central olfactory cortical structure with extensive connections to the olfactory bulb, and to the rest of the brain through the piriform cortex1. Utilizing a transgenic rat that expresses an eGFP-vasopressin fusion gene2, we detected previously undescribed populations of vasopressin neurones within the AON. These cells are found in groups in both the pars externa and along the edge of the outer plexiform layer of the pars principalis. To determine the neurochemical composition of the vasopressin expressing cells we used fluorescent immunohistochemistry to label GFP or vasopressin in combination with specific antibodies against the neurotransmitter, GABA and glutamate, and the calcium sensing proteins, calretinin and calbindin-D-28K. In addition, we examined whether AON vasopressin expressing cells express vasopressin receptors. Our data show that the vasopressin cells in the AON are immuno-positive for GABA and calbindin-D-28K and express both the V1a and V1b receptors receptor subtype. As vasopressin has been established as an important regulator of mammalian social behaviour3, we quantified immediate early gene (Egr1) induction in vasopressin (eGFP) neurons in the AON in male and female rats exposed to social and non-social odours, and in lactating female rats after a maternal aggression test. The number of cells expressing both Egr1 and vasopressin in the lateral subregion of the AON increased after exposure to a conspecific juvenile, but not after exposure to a non-social odour. On the other hand, the number of double-labelled cells was significantly depressed in dams exposed to a maternal aggression test. These data suggest that vasopressin in the AON is involved in the processing of socially relevant odour cues.

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PL2

Properties, Performance and Plasticity of Identified Oxytocin and Vasopressin Neurons in vitro

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The neurohypophysial hormones oxytocin (OT) and vasopressin (VP) originate from hypothalamic neurosecretory cells in the paraventricular and supraoptic (SON) nuclei. The pace and pattern of action potentials arising from these neurons determine the timing and quantity of peripheral hormone release. We have used immunochemical identification of biocytin-filled SON neurons in hypothalamic slices to uncover differences between OT and VP neurons in membrane and synaptic properties, firing patterns, and plasticity during pregnancy and lactation. Here we summarize some of our recent findings from this approach: 1) VP neurons more often exhibit slow (sDAP) and fast (fDAP) depolarizing afterpotentials and phasic bursting activity. The fDAP appears related to a transient receptor potential (TRP) channel type TRPM4 and/or TRPM5, both of which, from immunochemical studies, are more localized to VP neurons, and especially, to their dendrites. Both mRNAs are found in the SON, but single cell RT-PCR suggests TRPM4 might be the more prominent channel. Phasic bursting in VP neurons is little influenced by spontaneous synaptic activity in slices, being shaped largely by intrinsic currents. 2) The firing pattern of OT neurons ranges from irregular to continuous, with the coefficient of variation determined by randomly distributed, spontaneous GABAergic inhibitory synaptic potentials (sIPSCs). These sIPSCs are 4-5 fold more frequent in OT vs. VP neurons, and some of this difference relates to a tonic suppression of sIPSCs in VP neurons by cannabinoids. 3) Both cell types express Ca++-dependent afterhyperpolarizations (AHPs), including an apamin-sensitive, medium duration AHP and a slower, apamin-insensitive AHP (sAHP). In OT neurons, both AHPs are specifically enhanced during pregnancy and lactation, and during pregnancy, the plasticity of the sAHP is mediated by central OT receptors. Supported by NIH grants NS23941 (WEA) and HL093728 (RT).

A Scientific Life Fully Realized: Glenn I. Hatton, Ph.D.

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Neuroscience, and particularly neuroendocrinology, lost a great friend and pioneering investigator with the death of Glenn I. Hatton on January 16, 2009, following a brief illness from pancreatic cancer. At the time of his passing, Glenn was a Distinguished Professor in the Department of Cell Biology and Neuroscience at University of California, Riverside, where he had served as Chair, and as founding Director of the Neuroscience Program from 1992-2002. He had recently celebrated his 74th birthday.

Glenn hailed from Chicago, Illinois, and received his B.S. from North Central College, Naperville, Illinois in 1960 in Psychology. He then studied under Professor Larry O'Kelly at the University of Illinois, Urbana, where he received M. A. (1962) and Ph.D. (1964) degrees in Psychology. After a very short postdoc, Glenn became Assistant Professor of Psychology at Michigan State University in East Lansing in 1965, where he would remain for 26 years and become Distinguished Professor in 1986. He also served as Director of MSU's fledging Neuroscience Program from 1978-1991.

Glenn's career began with behavioral studies investigating the mechanisms of thirst and drinking. His curious and expansive nature led him into neurophysiological and neuroanatomical studies, under the tutelage of an early collaborator, John I. Johnson. In the early 1970s Glenn began to apply these methods to studies of oxytocin and vasopressin cells in the supraoptic and paraventricular nucleus, and it was in the study of these magnocellular neurosecretory neurons that Glenn was to make many seminal and lasting contributions, from over 150 peer-reviewed articles and many insightful reviews and chapters.

With Charles Tweedle, Glenn published landmark papers in 1976 and 1977 demonstrating a dynamic morphological relationship between glia and neurons, a relationship very sensitive to the physiological state of the animal. This foray into quantitative electron microscopy served as foundation for a series of papers on neuro-glial relationships in both hypothalamus and neurohypophysis, and crystallized Glenn's profound interest in how neurons and glia intercommunicated in order to shape their structure and function.

In 1977 Glenn took a sabbatical with Gary Lynch at the University of California, Irvine to learn the brain slice technique, and soon after his lab was first to apply this technique to the hypothalamus in 1978 Glenn enjoined collaboration with Ed Dudek, whose lab then made the first published intracellular recordings from neurosecretory neurons in the rat hypothalamic slice preparation and the first reports of dye coupling in these neurons. Glenn continued developing this technique on sabbatical with Bill Mason at the Babraham Institute in Cambridge in 1981, where, among other things, he studied the synaptic, cholinergic excitation of phasic neurons. This would be the first of two sabbaticals Glenn would spend in England, a country, and whose people, he greatly admired. On his second visit a few years later, he and John Bicknell demonstrated that vasopressin and adrenaline directly altered pituicyte morphology and calcium regulation. Fogarty and Guggenheim Foundation Fellowships supported Glenn's time in Cambridge, where he became a member of Corpus Christi College and a University of Cambridge Senior Scholar.

Electrophysiological studies characterized the Hatton lab until the end, and along the way produced many revolutionary, sometimes controversial findings. A short list, in no way comprehensive, includes evidence that: 1) dye-coupling (suggestive of electrotonic communication) among neurosecretory neurons was prominent, modulated by transmitters, sex steroids, and physiological state; 2) phasic bursting activity characteristic of vasopressin neurons occurred independent of synaptic transmission; 3) the degree of intrinsic buffering

by calbindin determined whether magnocellular neurons firing phasically or not; 4) the slow depolarizing afterpotential underlying phasic activity resulted from a calcium-dependent decrease in a potassium current; 5) histamine released from tuberomammillary neurons mediated fast, H1-receptor-mediated EPSPs and fast, H2-receptor mediated IPSCs on supraoptic neurons; and 6) acetylcholine mediated fast EPSPs via alpha-7 nicotinic receptors. Most recently, in a final and amazingly productive period, Y-F Wang and Glenn provided intriguing evidence that oxytocin promoted milk-ejection-like bursting in supraoptic neurons by way of complex, second messenger pathways involving actin mobilization, and astrocyte remodeling.

Glenn's career had many marks of distinction. He was continuously funded from the National Institutes of Health, from which he was twice awarded the prestigious Jacob Javits Award as well as a Career Development Award. He served as president of the Association of Neuroscience Departments, on many NIH and NSF panels, and on journal editorial boards. He was very proud to have edited, with Vlad Parpara, the remarkably successful book: Glial-Neuronal Signaling (Kluwer, Amsterdam, 2004).

Glenn was an exuberant scientist who never lost the zest for discovery or debate. He was fearless in approach, unguarded in opinion. He was an outstanding and demanding teacher, a critical yet supportive colleague. He loved family first. Wine and science followed, in no particular order. He is survived by his wife of 53 years, Patricia Dougherty Hatton, his children Jim, Bill, Chris, Jennifer and Tracy (Silla), his granddaughter Aubrey and his many friends, collaborators, colleagues, students, and postdocs.

Glenn Hatton: conjectures and refutations

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In “The Logic of Scientific Discovery”, Karl Popper declares that ‘Bold ideas, unjustified anticipations, and speculative thought are our only means for interpreting nature: our only organon, our only instrument for grasping her. And we must hazard them to win our prize. Those among us who are unwilling to expose their ideas to the hazard of refutation do not take part in the scientific game.” In 1976, Glenn Hatton warned his fellow electrophysiologists that we couldn’t take anatomy for granted, that the cellular architecture of the magnocellular nuclei and the neurohypophysis was dynamically regulated by physiological stimuli (1). In subsequent years he vigorously pursued ambitious theories about how changes in the contacts between magnocellular neurones and between magnocellular neurones and glial cells might explain some of the major unanswered questions about the physiological regulation of neurohypophysial hormone release, and tested those theories by innovative experiments (2). I will trace the origins and subsequent fate of some of Glenn’s boldest ideas.

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SS3

My tribute to Glenn's findings and concepts on function related plasticity in hypothalamus

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Glenn Hatton is one of the investigators who has taken up the challenge of elucidating the functional implications of dynamical neuronal glial interactions and in particular their consequences on oxytocin and vasopressin neuronal activity in the supraoptic nucleus of the hypothalamus. Glenn set out to determine if, to what extent, and in what ways, neuron-glia crosstalk occurs and his findings have given rise to major concepts that have influenced or provided the starting point of research projects of many scientists, including several of my own lab's studies. Among these I would like to emphasize the three following ones :

Neuron-glia interplay in the neural lobe. The particular role of classical neurotransmitters in shaping astrocyte morphology, as shown by Glenn's group (Bicknell et al., 1989; Hatton et al., 1991), has led my own laboratory to study if aged-related morphological changes in pituicytes result from changes in their innervation in the neural lobe.

Dynamic participation of astrocytes in osmoregulation by the hypothalamo-neurohypophysial system. Glenn's discovery of the presence of taurine in astrocytes (Decavel & Hatton, 1995) and pituicytes (Miyata et al, 1997) gave rise to the hypothesis that this GABA-like amino acid might be a factor contributing both to low excitability of supraoptic neurons under basal conditions (Hatton, 1999) as well as to peptide release from axon terminals (Miyata & Hatton, 2002; Song & Hatton, 2003). From the series of in vivo and in vitro experiments done in my lab in Montpellier, along with those of Glenn, emerged a clear picture of the role of taurine in the dynamics of glial-neuronal interactions in which release of this transmitter substance is initiated by physiological osmotic conditions and not simply a result of incoming neural signals (Hatton, 2004).

Factors involved in the generation and structure of oxytocin neuronal bursting. Over the last ten years, most of Glenn's studies were aimed at elucidating multiple aspects of the mechanisms underlying burst firing using in vivo and in vitro models (Wang & Hatton, 2004, 2005; Wang et al., 2006). Several important findings have emerged from these studies, each of them being linked to Glenn's high capacity to periodically review literature data, to summarize major findings, to emphasize current ideas, and then to address unsolved questions and propose new mechanisms. The major domains in which Glenn's and my own data have been mutually reinforcing were the autocontrol by oxytocin, the factors involved in the generation, structure and synchronisation of bursts and some of the cellular and molecular mechanisms underlying bursting.

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S3-1

FUNCTIONAL CONSEQUENCES OF VASOPRESSIN AND CORTICOLIBERIN RECEPTORS CO-EXPRESSION IN NATIVE AND HETEROLOGOUS MODELS

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In mammals, Vasopressin (VP) and corticoliberin (CRF) co-regulate ACTH and insulin release by acting in synergism at the pituitary and pancreas levels respectively. The molecular mechanisms involved remain partially unknown.
In this study, we first extend this notion to the bovine adrenal chromaffin gland. We showed that VP and CRF both stimulate catecholamines secretion and also act in synergism. Such potentiation may be related to modifications of second messenger cascade. Thus, we observed a clear potentiation of VP-stimulated Inositol Phosphates (IPs) accumulation by CRF and a weak but significant effect of VP on CRF-stimulated cAMP production.
To go deeper in these mechanisms, we transfected HEK293 cells with functional tagged V_{1b} and CRHR1 receptors .We first demonstrated by BRET and immunoprecipitation experiments that these 2 receptors heterodimerize in living cells. Then, we explored the potential modifications of V1b and CRHR1 receptors pharmacology upon receptor co-transfection. We found that CRF may alter VP binding at high doses. More interestingly, we showed that VP potentiated the CRF-stimulated cAMP accumulation. This effect was dose- dependent, receptor-mediated since antagonized by a selective V_{1b} antagonist and partially due to PKC activation. Yet, 30% of this potentiation effect was insensitive to a full PLC antagonist suggesting another mechanism of synergy. CRF may also weakly but significantly potentiate VP-stimulated IPs accumulation.
In conclusion, we show that VP/CRF potentiation seems to be a general phenomenom in native tissues. We also bring evidence that, beside second messengers crosstalk, heterodimerization of V1b/CRHR1 receptors may also be involved in such a phenomenom.

INTRACELLULAR ACTIVATION OF VASOPRESSIN V2 RECEPTOR MUTANTS IN NEPHROGENIC DIABETES INSIPIDUS BY NON-PEPTIDE AGONISTS.

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To prevent dehydration, binding of the peptide hormone vasopressin to its type-2 receptor (V2R) in kidney triggers a cAMP-mediated translocation of Aquaporin-2 water channels to the apical membrane, resulting in water reabsorption. Mutations in the V2R gene lead to Nephrogenic Diabetes Insipidus (NDI), because the, often intrinsically-functional mutant V2 receptors, are misfolded and retained in the endoplasmic reticulum (ER). Since plasma membrane expression is thought to be essential for V2R activation, cell permeable V2R antagonists were used to rescue cell surface expression of V2R mutants, but then require displacement by vasopressin for activation. To circumvent displacement, we analyzed whether non-peptide cell-permeable agonists can induce function restoration of V2R mutants in NDI.

We stably expressed wild-type V2R or several V2R mutants in NDI in polarized kidney cells and analyzed them for subcellular localization and glycosylation state of the V2R. Next, cells were treated with three cell permeable agonists and the effects of these compounds on receptor localization, maturation, cAMP production and ability to induce translocation of Aquaporin-2 were analyzed. We found that six out of seven V2R mutants are intrinsically functional. Whereas the mature wild-type V2R localized to the basolateral membrane, the mutants were immature and were retained inside the cell. Treatment with the non-peptide V2R agonists, but not vasopressin, activated NDI-causing V2R mutants at their intracellular location, but did not induce their maturation or translocation to the basolateral membrane. Activation of the intracellular receptors induced a cAMP response sufficient to induce the translocation of aquaporin-2 to the apical membrane. Moreover, in contrast to plasma membrane V2R, degradation of intracellular V2R mutants is not increased by their activation.

Our data reveal that plasma membrane G protein-coupled receptors (GPCRs) can also be activated intracellularly, and indicate that non-peptide agonists constitute highly-promising therapeutics for diseases caused by retained, but intrinsically-functional GPCRs in general, and NDI in particular.

S3-3

Moving mechanisms of aquaporin-2 water channel

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Aquaporin-2 (AQP2) is a recycling water channel in the kidney. AQP2 translocation to the apical membrane is a key event for water reabsorption and regulates the final volume and concentration of urine. Its impairments result in various water balance disorders including nephrogenic diabetes insipidus. AQP2 relocation is under the control of vasopressin. This hormone activates PKA, which in turn phosphorylates AQP2. But how this phosphorylation induces AQP2 movement has been completely unknown. We have recently discovered the direct mechanism, which drives AQP2 movement to the apical membrane. Surface plasmon resonance (SPR) measurements show specific binding of AQP2 to G-actin in reconstituted liposomes, which is negatively regulated by PKA phosphorylation. Dual color fluorescence cross-correlation spectroscopy (FCCS) reveals local AQP2 interaction with G-actin both in the subapical region and on the apical membrane in the AQP2 trafficking pathway in live cells at the resolution of single molecule. Coimmunoprecipitation, cosedimentation and pyrene-actin assays show the role of AQP2 phosphorylation in its interactions with actin and TM5b, and in actin dynamics. Under basal conditions, AQP2 binds to G-actin and F-actin stabilized by tropomyosin 5b (TM5b) forms a barrier inhibiting translocation of AQP2 toward the apical membrane. Vasopressin-triggered cAMP signaling and phosphorylation at serine 256 of AQP2 release AQP2 from G-actin and promote AQP2 association with TM5b, which sequesters TM5b from F-actin and destabilizes F-actin network, allowing efficient movement of AQP2. Knockdown and overexpression of TM5b confirm its role in apical trafficking of AQP2 through apical actin reorganization in the trafficking pathway. These findings indicate a novel mechanism of channel protein trafficking, in which the channel protein itself critically regulates local actin reorganization to initiate its movement. Because trafficking of many other channels is regulated by phosphorylation, and because many channels interact with actin, it is speculated that other channels may also promote their own relocation through this scheme. Our present findings also suggest TM5b is an appropriate therapeutic target for nephrogenic diabetes insipidus.

Vasopressin Signaling in the Renal Collecting Duct: Advances from Protein Mass Spectrometry

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Arginine vasopressin (AVP) regulates water, urea, and NaCl transport in the kidney via V2 receptor-mediated signaling processes. At the protein level, signaling in any cell consist of a few basic processes that are manifest as changes in 1) abundances of individual proteins; 2) post-translational modifications at specific sites; 3) protein localization in the cell; and 4) binding interactions with other macromolecules. We are developing liquid chromatography (LC)-tandem mass spectrometry (MS/MS) techniques for quantification of each of these processes and applying them to the study of AVP signaling in native renal inner medullary collecting duct (IMCD) cells (http://dir.nhlbi.nih.gov/labs/lkem/rm/proteomics_db.asp). Large-scale phospho-protein profiling has revealed over 600 phosphorylation sites in IMCD including four AVP-regulated sites in the water channel aquaporin-2 (AQP2) and two AVP-regulated sites in the collecting duct urea transporter UT-A1. Among the proteins whose phosphorylation is regulated by AVP in the IMCD is myosin regulatory light chain (MRLC), which we have shown to be part of a non-canonical signaling pathway involving aperiodic intracellular calcium spikes, calmodulin activation, activation of myosin light chain kinase and non-muscle myosin II-mediated AQP2 translocation. Additional studies have probed binding partners for phosphorylated forms of AQP2, revealing selective binding of Hsp70-5 to the Ser256 phosphorylated AQP2. Apical surface biotinylation of IMCD cells has revealed an unusual abundance of proteins with COOH-terminal PDZ ligand motifs including AQP2, and has demonstration three bound PDZ domain proteins. Immunogold electron microscopy reveals that phosphorylation of AQP2 at Ser269, part of its PDZ ligand motif, results in retention of AQP2 in the apical plasma membrane. We propose a model of AVP-mediated regulation of AQP2 localization in which Ser269 phosphorylation modifies PDZ interactions that affect plasma membrane retention.

S4-1

Agonist activation and oxytocin receptor trafficking: role of different G-proteins

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As in the case of most G-protein coupled receptors (GPCRs), agonist stimulation of human oxytocin receptors (OTRs) leads to desensitization and internalization; however, little is known about the subsequent intracellular OTR trafficking, which is crucial for re-establishing agonist responsiveness. We examined receptor resensitization by first using HEK293T cells stably expressing human OTRs. Upon agonist activation, the receptors were almost completely sequestered inside intracellular compartments but are not sorted to degrading organelles. Instead, binding and fluorescence assays showed that almost 85% of the receptors had returned to the cell surface after four hours, by which time cell responsiveness to the agonist was also completely restored. Finally, investigations of receptor recycling pathways showed that OTRs localize in vesicles containing the Rab5 and Rab4 small GTPases (markers of the “short cycle”), whereas there was no co-localization with Rab11 (a marker of the “long cycle”) or Rab7 (a marker of vesicles directed to endosomal/lysosomal compartments). Taken together, these data indicate that OTRs are capable of very efficient and complete resensitization due to receptor recycling via the “short cycle” (Conti et al, 2009, AJP-EM).
The oxytocin receptor (OTR) is a promiscuous G-protein coupled receptor that couples to both Gαq and Gαi and whose stimulation leads to the activation of different intracelluar signaling pathways. OT –derived peptides that activate selectively either the Gαi or Gαq pathways were characterized in our laboratory (Reversi et al 2005 JBC). In order to assay the capability of these analogs to promote receptor internalization we developed two fluorescent agonists: dLVT-Alexa568 and atosiban-Alexa568). After stimulation of the cells with dLVT-Alexa568, that promotes both OTR/Gαq and OTR/Gαi coupling, we observed internalization of both the receptor and the fluorescent peptide. The two molecules were initially colocalized in vesicles, but this colocalization was lost after two hours when the receptor and the ligand were found in different vesicles, suggesting that they followed different trafficking pathways. On the contrary, atosiban-Alexa568, a selective OTR/Gαi, did not lead to any change in receptor localization at the plasma membrane even after one hour of stimulation. Since OTRs are expressed in the CNS in neuronal and glial cells, we finally used a neuronal cell line (the mouse neuroblastoma Neuro2A cells) transiently tranfected with human OTR to further investigate OTR trafficking. Both the OTR-EGPF and OTR tagged at its N-terminus with an HA epitope were localized at the plasma membrane as expected, but, again, receptor internalization was observed only after stimulation with dLVT-Alexa568. All together, these data suggest that Gαq activation plays an important role in OTR internalization.

Vasopressin V1a receptor agonists in vasodilatory shock: from bedside to bench and back

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We have shown that a deficiency in arginine vasopressin (AVP) and hypersensitivity to the vasopressor effect of this hormone are hallmarks of vasodilatory hypotensive states such as septic shock and late-phase hemorrhagic or cardiogenic shock. Low-dose intravenous infusion of AVP restores blood pressure in these conditions. Recently, the first large multi-center randomized, blinded, controlled trial of AVP vs. norepinephrine infusion in septic shock (VASST: Vasopressin in Septic Shock Trial) showed a possible beneficial effect for AVP in patients with mild to moderate septic shock. AVP is a mixed agonist at the V1a and V2 receptors. Activation of the V1a receptor mediates its vasopressor action. Activation of the V2 receptor promotes vasodilation and coagulation, effects that are undesirable in septic shock. We have thus specifically designed a novel AVP analog for the treatment of vasodilatory shock, FE 202158, which is a selective agonist at the V1a receptor. FE 202158 remains short-acting like AVP, which allows for a rapid rise to steady-state plasma concentration, dose titration, and rapid onset and offset of action by intravenous infusion, all essential features for its intended uses in critical care medicine. In addition, FE 202158 displayed a novel anti-vascular leak effect in animal models of septic shock. Currently, FE 202158 is entering phase II clinical trial (first-in-patient) for early septic shock.

S4-3

Discovery of novel neuropeptides NERPs that suppress vasopressin secretion

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Neuroendocrine regulatory peptide (NERP) -1 and NERP-2 are novel amidated peptides identified from secretory peptides produced by human medullary thyroid carcinoma TT cells (J Biol Chem 282: 26354–26360, 2007). NERPs are derived from distinct regions of the neurosecretory protein VGF that was originally identified as a product of a nerve growth factor-responsive gene in PC12 cells. NERPs were abundant in the paraventricular and supraoptic nuclei of the rat hypothalamus and colocalized frequently with vasopressin, but rarely with oxytocin. NERPs dose-dependently suppressed vasopressin release induced by icv injection of hypertonic NaCl or angiotensin II in vivo. NERPs also suppressed basal and angiotensin II-induced vasopressin secretion from hypothalamic explants in vitro. Bioactivity of NERPs required carboxy-terminal amidation. NERP-2, but not NERP-1, enhanced food intake, body temperature, oxygen consumption, and locomotor activity. These findings suggest that NERPs are novel modulators with diverse neuroendocrine functions.
(This study was done in collaboration with Drs. Sasaki and Minamino at National Cardiovascular Center Research Institute; Dr. Takao at Osaka University; Dr. Shioda at Showa University School of Medicine.)

STRUCTURAL EVOLUTION OF NEUROHYPOPHYSIAL HORMONES, THEIR PRECURSORS AND THEIR RECEPTOR

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Abstract

Most physiological functions can now be described as spatiotemporal networks of cascades of protein machineries in which specific protein-protein recognition depends upon respective conformations. The so-called neurohypophysial hormones and their specific receptors, largely distributed among the animal kingdom, build up a crucial machinery within the signal transducing pathway.

Up to date 13 neuropeptides from some 80 vertebrate species and 6 invertebrate homologous peptides have chemically been characterized evoking the existence of an ancestral gene antedating the animal divergence, 700 million years ago. All these peptides possess nine residues, a disulfide bridge in position 1-6, two hydrophobic residues in positions 2, 3, two polar residues in position 4, 5, a Pro in position 7 and a C-terminal glycinamide, suggesting similar foldings and conformations. They derive through specific processing from homologous precursors (110-150 residue long) in which there are linked to a 7-disulfide bridge-containing “neurophysin” domain. All encoding genes have 3 exons displaying different evolutionary rates. In vertebrates, vasotocin is the single peptide found in the most primitive Cyclostomes, but in all higher classes two similar peptides exist, suggesting a duplication of the vasotocin gene before the rise of bony fishes. On the basis of structure and activity, two paralog evolutionary lineages have been traced, a basic vasopressin line: vasotocin (non-mammals)-Arg/Lys vasopressin (mammals) and a neutral oxytocin line: isotocin (bony fishes), mesotocin (non-mammalian land vertebrates) oxytocin (placental mammals). Evolution operates essentially by punctual substitutions in positions 3, 4 and 8 through neutral or selective mechanisms. Neurophysin domains, encoded by the last 2 exons, show particular subdomain evolutionary rates.

Three types of vasopressin receptors V1a, Vib, V2 and one type of oxytocin receptor have been identified in mammals. They trigger particular signal transducing cascades through heterotrimeric G proteins (Gq or Gs) and control distinct physiological functions, namely vascular tone, corticotropin secretagogue, antidiuresis and milk ejection, respectively. Corresponding evolutionary lineages exist across vertebrates. All belong to the superfamily of 7-transmembrane G protein-coupled receptors. Crystal structures of two prototypes, rhodopsin (Palczewski, 2000) and b2-adrenoreceptor (Kobilka, 2007) are known, allowing computational homology modeling for vasopressin receptors. Speculative localization of ligand- and G protein-binding sites suggest an allosteric functioning of the signaling machinery due to conformational flexibility.

>From a unique ancestral vasotocin receptor AVT-R in Cyclostomes, three successive gene duplications led to the four vasopressin Vla, Vib, V2 and oxytocin receptors in mammals. Co-evolution of hormones and receptors has required multiple structural adaptations in each other through genetic mechanisms.

ST2

Oxytocin's centenary: Discovery, synthesis, drugs, current and future indications

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Sir Henry H Dale discovered in 1909 that a posterior pituitary extract could contract the uterus and named it oxytocin from the Greek "quick birth". Thereupon sterilized pituitary extracts entered quickly into the gynaecological routine for labour induction and prevention of postpartum haemorrhage.
Vincent du Vigneaud isolated and synthesised oxytocin in 1953 and was honoured with the Nobel Prize. In parallel to this scientific progress, the pharmaceutical company Ferring was founded and specialised in isolation and synthesis of peptides for gynaecology and urology. In the 1990s, the oxytocin receptor was identified, and consequently knockout mice for oxytocin and its receptor became available.
Carbetocin is a long-acting oxytocin analogue indicated for uterine atony; other non-peptide agonists reached development. Atosiban is a mixed vasopressin V1a and oxytocin antagonist for treatment of imminent preterm birth; barusiban is a selective oxytocin antagonist. Further experimental peptide antagonists were synthesised, and non-peptide antagonists were developed for oral administration or central indications.
Following the classical gynaecological indications, recently central roles of oxytocin became obvious. Pair bonding and attachment has been characterised in prairie voles. Oxytocin seems to be involved in autism. Trust and social interaction is facilitated by oxytocin. However also as a peripheral neuropeptide, oxytocin plays various roles such as in the cardiovascular system.
A hundred years of oxytocin in science will be presented, and an overview will be given on existing oxytocin agonists and antagonists as well as on the spectrum of possible future indications.

Intrinsic versus synaptic control in magnocellular neurones?

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The neuropeptides vasopressin (VP) and oxytocin (OT) respectively synthesised in VP and OT hypothalamic magnocellular neurones, play a key role in body-fluid and cardiovascular homeostasis, and during reproduction. Under normal conditions, most VP and OT neurones display a slow irregular firing pattern. On the one hand, during a hyperosmotic stimulus, VP neurones evolve a phasic activity composed of bursts of action potentials (APs) alternating with silent periods (1, 2). On the other hand, preceding each foetus expulsion or milk ejection, OT neurones display a high frequency burst of APs (2). Because they optimise the efficiency of stimulus-secretion coupling, understanding the mechanisms underlying these specific patterns is of crucial importance. In hypothalamic acute slices, phasic activity was shown to be linked to an intrinsic property of VP neurones, the depolarizing after-potential. However, we showed that in juvenile rat hypothalamic slices maintained in non stationary organotypic culture, both phasic activity and high frequency bursts depend mainly on afferent glutamatergic activity. However, the strength of this glutamatergic control can be adapted according to physiological needs. For example, OT, VP, steroids or opiates are able to modulate the amplitude, the frequency and/or the decay of EPSCs/EPSPs. Also, during lactation, astrocytes may influence synaptic efficacy through a decrease of synaptic glial coverage leading to a change in glutamate clearance (3) and through the release of D-serine, the coagonist of NMDA receptors in the supraoptic nucleus (4).
We conclude that the specific firing in OT and VP magnocellular neurones mainly results from distinct robust external glutamatergic controls, adequately regulated by intrinsic and extrinsic factors, to finely tune OT and VP secretion.

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S5-2

Dehydration-induced plasticity in autocrine modulation of vasopressin neuron activity

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Vasopressin (anti-diuretic hormone) secretion from the posterior pituitary gland is largely determined by the pattern of action potential (spike) discharge of vasopressin neurons in the hypothalamic supraoptic and paraventricular nuclei. In recent years it has become clear that multiple autocrine feedback mechanisms restrain the activity of vasopressin neurons. Many of these feedback mechanisms are generally inhibitory and might function to prevent over-excitation. However, we have demonstrated that some feedback drives vasopressin neurons towards adopting a phasic spike discharge pattern, which is most efficient for vasopressin secretion from the posterior pituitary gland. Phasic spike discharge is characterized by alternating periods of activity (bursts) and silence. One of the best-characterized mechanisms that promote phasic activity is endogenous kappa-opioid inhibition of the afterdepolarization (1), which contributes to burst termination (2).

During dehydration, vasopressin neurons increase their firing rate, which increases vasopressin secretion from the posterior pituitary gland to reduce water loss in the urine. Our recent in vivo extracellular single unit recordings show that microdialysis administration of the specific kappa-opioid receptor antagonist, nor-binaltorphimine, into the supraoptic nucleus is equally effective at increasing intraburst firing rate of phasic neurons in dehydrated rats as in non-dehydrated rats. Therefore, endogenous kappa-opioid feedback inhibition of phasic activity appears to be proportionately increased during dehydration. Hence, even under stimulated conditions, endogenous kappa-opioid feedback promotes the adoption of a phasic firing pattern by some vasopressin neurons.

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Stress-induced behavior and plasma oxytocin levels during development are regulated dually by breast milk and hypothalamic ADP-ribosyl cyclase of CD38 in mice

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Oxytocin (OT), a neurohormone involved in reproduction, also plays a critical role in social behavior from rodents to humans and is related to autism. CD38, a transmembrane protein, is highly expressed in the hypothalamus (1). Neuronal roles of CD38 have been recently revealed (2). CD38-dependent regulation of OT secretion is critical for social behavior in adult mice. But it has not been examined in infants or during development. To assess the above question, we used separation from the mouse dam in 7-day old pups. During such isolation stress, locomotor activity was higher in CD38 knockout ($CD38^{-/-}$) pups than in wild-type controls ($CD38^{+/+}$). The frequency of ultrasonic vocalization (USV) was lower in $CD38^{-/-}$ pups than in $CD38^{+/+}$ pups (3). However, the difference between the two genotypes seems to be less severe than those in OT knockout or OT receptor knockout mice. To explain this, we measured plasma OT levels. The OT level was not lower in $CD38^{-/-}$ pups during the period from 1–3 weeks after birth, but was significantly reduced after weaning (≤3 weeks), as reported before (1). ADP-ribosyl cyclase activities in the hypothalamus and pituitary were markedly lower from 1 week after birth in $CD38^{-/-}$ mice and were consistently lower thereafter to the adult stage (2 months old). In addition, we found that the mammary gland and breast milk of lactating dams were rich in OT. These results demonstrate that the reduced severity of behavioral abnormalities in $CD38^{-/-}$ pups is due to high levels of plasma OT taken from the dam's milk. These results suggest that CD38-dependent secretion of OT into the brain from hypothalamic neurons is important for social behavior and that OT levels are dually regulated before the critical switching time (at weaning 3 months after birth) to assist in social brain development.

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PL4

Vasopressin and oxytocin in pregnancy: Water channels, ion transporters, and receptor antagonists

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Pregnancy is associated with systemic arterial vasodilation in the first trimester of gestation. This relative arterial underfilling leads to a compensatory rise in cardiac index and activation of the neurohumoral axis including stimulation of the non-osmotic stimulation of arginine vasopressin (AVP). The rise in plasma AVP leads to a decline in plasma osmolality of approximately 10 mOsm/kg H2O in normal pregnancy. This hypoosmolar affect of pregnancy is due to an AVP-mediated increase in aquaporin 2 expression and trafficking to the apical membrane of principal cell of the collecting duct. The increase membrane water permeability then results in enhanced solute-free water transport in the renal collecting duct. Hypoosmolality also occurs during induction of labor with oxytocin. Oxytocin exerts its contractible effect on the uterus via oxytocin receptors. Studies, however, have shown that the oxytocin-related hypoosmolality is mediated by the V2 vasopressin receptors in the renal collecting duct independent of any oxytocin receptor. Thus, oxytocin-induced hypoosmolality during labor induction could be treated with a V2 receptor antagonist as the oxytocin receptor-mediated uterine contraction persists.

Dehydration switches control of sympathetic activity from forebrain to hindbrain

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Using the in situ working heart-brainstem-hypothalamus preparation (1), we have investigated the brain regions responsible for the increase in sympathetic nerve activity (SNA) resulting from the hyperosmolality caused by 3 days of fluid deprivation (dehydration, DH). In euhydrated (EH) rats (290 mOsmol/kg perfusate), systemic application of Losartan (angiotensin II type 1 AT_1 receptor antagonist), and subsequent pre-collicular transection (to remove the hypothalamus) significantly reduced thoracic SNA. In contrast, in DH rats (340 mOsmol/kg perfusate) Losartan, subsequent pre-collicular and pontine transections failed to reduce SNA. However, transection at the medulla-spinal cord junction massively reduced SNA. In intact DH, but not EH rats, reversible inactivation of cNTS using isoguvacine, (a $GABA_A$ receptor agonist), significantly reduced baseline SNA. These data indicate that in the EH rat, baseline SNA is dependent on both the hypothalamus and AT_1 receptors. However, following chronic dehydration, the control of SNA transfers to the medulla oblongata, particularly the cNTS.

Dehydration increases expression of AP1 transcription factors in the cNTS, particularly FosB/ΔFosB (Ji et al, 2007). To test the hypothesis that AP1 activity is responsible for dehydration-induced functional plasticity, we chronically blocked the activity of these transcription factors using a viral vector expressing a dominant negative FosB (Ad-CMV-IRES-eGFP-dnFosB) injected into the cNTS 5-7 days prior to 3 days water deprivation. In these animals, isoguvacine inactivation of the cNTS was ineffective. In addition, in DH rats in which AP1 was blocked in the cNTS, SNA was now decreased after pre-collicular transection, a response that was similar to that seen in control EH rats. Thus the dehydration-induced switch in the control of SNA from hypothalamus to brainstem seems to be mediated by activation of AP1 transcription factors, including FosB, in the cNTS. If AP1 activity is blocked in NTS during dehydration, then sympathetic activity control reverts back to forebrain regions. Microarray analysis is currently being used to identify potential AP1 target genes.

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S6-2

Translational Aspects of Arginine Vasopressin

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This review will focus on translational aspects of the neurohypophyseal hormone arginine vasopressin (AVP). In particular, we will address the molecular background behind different hereditary clinical phenotypes with disturbed AVP function. Autosomal dominant familial neurohypophyseal diabetes insipidus (adFNDI) is characterized by development of a severely deficient neurosecretion of AVP that appears after a postnatal period with completely normal AVP function. The genetic basis is now well characterized as the rate of new mutation reports has declined markedly. The mutation pattern, together with evidence from clinical, cellular, and animal studies, points toward a pathogenic cascade of events, initiated by protein misfolding, involving intracellular protein accumulation, and ending with degeneration of the AVP producing magnocellular neurons. Not all results, however, have been confirmatory and we present a novel silent adFNDI mutation challenging the 'toxic' hypothesis.
Molecular research has also provided an important tool in the occasionally difficult differential diagnosis of DI and examples of kindreds suspected of adFNDI but proving to have partial congenital nephrogenic DI (CNDI) are presented. Molecular evidence from such examples suggests that different CNDI mutations can create the same clinical phenotype through different intracellular mechanisms, e.g. defect V2 receptor translocation and defect receptor affinity.
Another condition associated with AVP dysfunction is monosymptomatic nocturnal enuresis where a circadian defect in AVP secretion results in nocturnal polyuria. Although this defect can be sporadic there are autosomal dominant forms and we present novel linkage to chromosome 17 in a kindred with clinical signs of nocturnal polyuria and excellent response to overnight DDAVP administration.
In conclusion, molecular research in adFNDI has provided an important tool in differential diagnosis of different DI forms and in the understanding of underlying pathogenetic mechanisms as well as an opportunity to perform presymptomatic diagnosis. Furthermore, molecular research may also provide important clues to a better understanding of the pathogenesis of a much more common water balance disorder, i.e. nocturnal enuresis.

Cellular mechanisms of progressive polyuria in a mouse model for familial neurohypophysial diabetes insipidus

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Familial neurohypophysial diabetes insipidus (FNDI), an autosomal dominant disorder, is mostly caused by mutations in the gene of neurophysin II (NPII), the carrier protein of arginine vasopressin (AVP). Previous studies suggest that loss of AVP neurons might be the cause of polyuria in FNDI. We have been analyzing knock-in mice expressing mutant NPII (Cys98stop) that causes FNDI in humans. The heterozygous mice manifested progressive polyuria as do patients with FNDI. Immunohistochemical analyses revealed that inclusion bodies that were not immunostained with antibodies for mutant NPII, normal NPII or AVP were present in the AVP cells in the supraoptic nucleus (SON), and that the size of inclusion bodies gradually increased in parallel with the increases in urine volume. Electron microscopic analyses showed that aggregates existed in the endoplasmic reticulum (ER) of AVP neurons in 1-month-old heterozygous mice, and that dilated ER filled with aggregates occupied the cytoplasm of AVP cells at 12 months. Analyses with in situ hybridization revealed that expression of AVP mRNA was significantly decreased in the SON in the heterozygous mice compared to that in wild-type mice. Counting cells expressing AVP mRNA in the SON indicated that polyuria had progressed substantially in the absence of neuronal loss. These data suggest that cell death is not the primary cause of polyuria in FNDI, and that the aggregates accumulated in the ER might be involved in the dysfunction of AVP neurons that lead to the progressive polyuria.

LS

Vasopressin Receptor Antagonists: Past, Present and Future

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Arginine vasopressin (AVP) is primarily responsible for regulating osmotic and volume homeostasis of body fluids. The AVP receptors responsible for these regulatory functions are the V_{1a} and V_2 subtypes. The V_{1a} receptors mediate vasoconstriction, glycogenolysis and platelet aggregation, while the V_2 receptors mediate renal free-water excretion and endothelial coagulation factor release. Increased AVP secretion leads to decreased free water excretion with resulting water retention. If the AVP secretion is inappropriate, it can cause a dilutional hyponatremia. Hyponatremia is the most common disorder of electrolytes encountered in clinical practice, occurring in up to 15% to 30% of both acutely and chronically hospitalized patients. Hyponatremia is important clinically because: 1) acute severe hyponatremia can cause substantial morbidity and mortality; 2) mortality is higher in hyponatremic patients with a wide range of underlying diseases; and 3) overly rapid correction of chronic hyponatremia can cause demyelination; 4) recent studies have suggested that even mild “asymptomatic” hyponatremia is accompanied by neurocognitive disturbances and gait instability. Optimal treatment strategies have not been well defined because of marked differences in symptomatology and clinical outcomes based on the acuteness or chronicity of the hyponatremia. AVP receptor antagonists have long been anticipated as a more effective method to treat hyponatremia by virtue of their unique effect to selectively increase solute-free water excretion by the kidneys (aquaresis). In 2005, the U.S. Food and Drug Administration (FDA) approved the first such agent, conivaptan, a combined V_{1a}-V_2 receptor antagonist, for short-term intravenous use, and in 2009 tolvaptan, a selective V_2 receptor antagonist, was approved for long-term oral use. Phase 3 trials indicate that both of these agents reliably reduce urine osmolality, increase electrolyte-free water excretion, and safely raise the serum sodium concentration. They are likely to become a mainstay of treatment of euvolemic and hypervolemic hyponatremia, thus heralding a new era in the management of hyponatremic disorders. This talk will describe the mechanism of action of AVP receptor antagonists, followed by a critical review the clinical data supporting the therapeutic use of AVP receptor antagonists, also known as “vaptans”, as alternatives or supplements to current therapies for the treatment of both acute and chronic hyponatremia. An algorithm for selecting appropriate therapy for hyponatremic patients based on presenting symptoms, including the use of vaptans, will be presented, and potential changes in the current treatment guidelines based on ongoing and future clinical studies will be discussed.

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Control of oxytocin neuron behavior perinatally: role of neuronal inputs from the uterus

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Oxytocin plays a role in determining the timing of birth. Oxytocin neurons coordinate parturition and milk ejection via peripheral pulsatile secretion, but they are also responsible for the exhibition of a whole suite of parental behaviors at this time. At birth, initial signals from the contracting uterus, mediated by a brainstem noradrenergic pathway, activate oxytocin neurons as measured by increased firing rate, immediate early gene (Fos) co-expression and dendritic oxytocin release. It is known that oxytocin autoregulates its own release within the SON via local positive feedback, and may presynaptically control local noradrenaline release, and together the noradrenaline and extracellular oxytocin evidently contribute to the specialized patterning of oxytocin neuron activity and its resulting physiological consequences. We are investigating the underlying mechanisms that locally control oxytocin and noradrenaline interaction within the supraoptic nucleus (SON). As well as release, oxytocin action also depends upon other factors: i.e. the oxytocin receptor and oxytocin extracellular degradation. Oxytocin receptor mRNA expression dynamically increases in the SON (but not PVN) on the day of birth, rapidly decreasing again with 12h. Oxytocin receptor-positive neurons in the SON also co-express Fos at birth, therefore the sensitivity of SON neurons to oxytocin likely increases perinatally. Furthermore, the activation of oxytocin sensitive neurons and their oxytocin receptor mRNA increases in the brainstem noradrenergic nuclei at birth, also indicating a potential role for oxytocin in controlling this input perinatally and reinforcing the importance of oxytocin receptor patterns of expression on behaviors. Oxytocin content in extracellular fluid depends upon both release and degradation- we revealed that the enzyme which degrades oxytocin, oxytocinase (placental leucine aminopeptidase) is expressed in the SON, including within oxytocin neurons. Simultaneously with increased oxytocin receptor expression, oxytocinase activity within the SON decreases compared to late pregnancy. So there is also dynamic control of extracellular oxytocin availability and this presumably contributes to dynamic control of oxytocin action perinatally. Together the perinatal increase in oxytocin receptor expression and oxytocin availability may initiate oxytocin neuron activity, perhaps via interaction with noradrenaline, and coordinate onset of parturition.

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S7-2

Oxytocin, vasopressin and affiliative behavior: neural circuitry, genetics and experience.

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Oxytocin (OT) and vasopressin (AVP) modulate various aspects of affiliative behavior, including parental nurturing and social attachment. Variation in OT and AVP systems contributes to both inter- and intra-species variation in social behavior. We have been using vole species with differing social organizations to investigate the roles of the OT and AVP systems in the regulation of affiliative behavior. In the socially monogamous female prairie vole, OT receptor (OTR) activation in the nucleus accumbens (NAcc) stimulates alloparental behavior and pair bond formation. In vivo microdialysis studies suggest that mating stimulates OT release within the NAcc, and infusion of an OTR antagonist into the NAcc blocks both alloparental behavior and pair bonding (1). The NAcc of rodents has a conserved innervation of large caliber fibers packed with OT-immunoreactive dense core vesicles that most likely originates from magnocellular hypothalamic OT neurons (1). This organization of forebrain OT projections may provide a mechanism of coordinated central and peripheral release under the appropriate circumstances (2). In contrast to the conserved pattern of OT fibers in the NAcc, OTR binding in NAcc varies strikingly across species, with prairie voles having high binding densities while non-monogamous rodents have little or no binding (2). In male prairie voles, AVP stimulates the pair bond formation, and variation in V1a receptor (V1aR) distribution to contributes variation in social behavior. A polymorphic microsatellite DNA element in the promoter of the V1aR gene (avpr1a) is associated with variation in V1aR binding, and the ability to form a pair bond. Similar microsatellite variability has now been associated with AVPR1A gene expression in the human brain as well as with altruistic and pair bonding behavior in humans (See 3 for a review). Finally, early life social experiences can impact adult social relationship quality and OT and AVP systems. Prairie voles raised by both a mother and a father develop pair bonds much quicker, and display more alloparental behavior than those raised by a mother only (4). Furthermore, rearing condition affects the number of OT-immunoreactive neurons in the hypothalamus. Interestingly, women who were abused or neglected in childhood have lower levels of OT in the CSF compared to control subjects (5). Thus both genetic variation as well as early life experience can contribute to variation the OT and AVP systems, which in turn may contribute to variation in social behavior.

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Brain arginine vasopressin is an important regulator of maternal care and aggression

Oliver J. Bosch, Inga D. Neumann

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Maternal care and aggression are important social behaviors of the lactating mother to secure the well-being and survival of the offspring regulated by oxytocin and prolactin. We will demonstrate that brain arginine vasopressin (AVP), which has mainly been associated with male social behaviors so far, is an additional regulator of different aspects of maternal behavior.
Using complimentary pharmacological and genetic approaches we assessed the role of AVP in maternal care and aggression. Viral vector mediated up-regulation of V1a receptors (AVP-R) within the medial preoptic area of lactating Wistar rats as well as chronic central AVP treatment improved maternal behavior, whereas local blockade of AVP-R expression via antisense oligodeoxynucleotides or central AVP-R antagonist (AVP-A) infusion impaired maternal care.
Our knowledge is complemented by studies in rats selectively bred for high (HAB) or low (LAB) anxiety-related behavior, which differ not only in anxiety, but also in various social including maternal behaviors. Lactating HAB dams spend more time on maternal care and are more maternally aggressive against a virgin intruder when compared with LAB dams. As HAB rats are characterized by an hyperactive brain AVP system, they provide a suitable model to study the involvement of AVP in maternal behavior. Thus, blockade of V1a receptors by icv AVP-A reduced both the level of anxiety and maternal care in HAB dams, whereas synthetic AVP heightened these behaviors in LAB dams. Brain AVP also promoted maternal aggression and local AVP release within the central amygdala correlated with the amount of aggressive behavior displayed during the maternal defence test. The high level of maternal aggression of HAB dams could be reversed by local retrodialysis of AVP-A, whereas AVP exerted the opposite effect in LAB dams.
In conclusion, our results show that the brain AVP system is importantly involved in the regulation of maternal care and aggression. Furthermore, HAB and LAB rats provide an ideal animal model to further investigate the involvement of AVP in aspects of maternal behavior.

PL5

The vasopressin gene and anxiety - from clue to causality

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The detection of AVP immunoreactivity and release patterns from dendrites/cell bodies/ axon terminals in distinct brain areas together with pharmacological studies suggested a role of this neuropeptide far beyond osmoregulation. Indeed, a variety of “from inside to outside” approaches demonstrated behavioral consequences of central AVP or AVP-receptor manipulations, including those on learning/memory, sociality and emotionality. Here, “from outside to inside” strategies are presented showing that extreme anxiety phenotypes in a robust, clinically relevant animal model are indeed associated with polymorphisms of the AVP but not oxytocin gene. First, focusing on emotionality and comorbid coping strategies, AVP is over-expressed in selectively bred HAB (high anxiety, passive coping) and under-expressed in LAB (low anxiety, active coping) rats and mice, respectively. Combining genotypic and expression data in an integrative genomics approach revealed polymorphisms likely to underlie differentiated expression profiles, including a promoter single nucleotide polymorphism (SNP), causing AVP over-expression, and a signal peptide SNP linked to a promoter deletion, both causing a deficit in AVP expression and processing. In addition to correlative evidence, causal associations between the SNPs and the phenotype could recently be shown in a freely-segregating F2 panel, generated from HABxLAB crosses. Thus, together with many other factors, polymorphisms in the AVP gene contribute to the variation of anxiety-related behavior up to psychopathology. Breeding rodents for extremes in emotionality seems to result in an accumulation of corresponding genetic polymorphisms that, in an outbred population, underlie behavioral variation, from resilience to disease susceptibility. While selective breeding is likely to attenuate the influence of variables such as maternal care, test conditions etc., even rigidly driven HAB mice, grown up in an enriched environment, may escape the constraints of inborn anxiety, highlighting interactions between genetic pre-disposition and environmental epigenomics in shaping the phenotype. Unlike copy number variation, the methylation status of the AVP gene seems to play a role in this context.

Genetic Analysis of Hypothalamic Neuron Development in Zebrafish

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Max Planck Institute for Medical Research, Heidelberg, Germany

The hypothalamus regulates diverse physiological responses critical for maintaining homeostasis. Reflecting the complexity of its function, the hypothalamus is composed of multiple nuclei, each composed of several neuronal cell types that form connections with many parts of the nervous system. Targeted gene knockouts in mouse have identified several transcription factors such as Otp, Sim1, Brn2, Arnt2, and Hmx2/3, which are required for the development of several hypothalamic neuronal types (reviewed in 1). However, the mechanism by which these factors regulate the generation of distinct neurons in hypothalamus is poorly understood. The zebrafish offers an excellent experimental system to dissect the molecular mechanisms of hypothalamic neuron development since it shares conserved developmental processes with mammals, yet contains much fewer neurons making it easier to analyze and manipulate these neurons. Using a zebrafish mutant in the homeodomain transcription factor Orthopedia (Otp), we previously demonstrated the essential role of Otp for the development of dopaminergic neurons in hypothalamus and posterior tuberculum in zebrafish and for the A11 dopaminergic group in mouse (2). Moreover, as in mouse mutant for Otp, zebrafish Otp mutant embryos lack neurons that produce corticotropin releasing hormone and isotocin (2-4) highlighting a striking conservation of its function across evolution. Our current experiments are aimed at dissecting the role of Otp in the specification of distinct hypothalamic neuron types. For in vivo analysis of hypothalamic neuron development, we have generated transgenic lines that label Otp expressing hypothalamic cells with green fluorescent protein (GFP).

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S8-2

Nonapeptides and the Evolution of Social Group Sizes in Finches: Touchstones for All Vertebrates?

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Proximate neural mechanisms that influence preferences for groups of a given size (or "sociality") are almost wholly unknown, although recent research demonstrates that the anatomy and functional properties of nonapeptide circuits are tightly coupled to sociality. In the highly gregarious zebra finch (Estrildidae: Taeniopygia guttata), blockade of nonapeptide receptors by an oxytocin (OT) antagonist significantly reduces time spent with large versus small groups, and familiar versus unfamiliar social partners. Opposing effects are produced by central infusions of mesotocin (MT; avian OT homologue). Most drug effects are female-specific. In contrast, blockade of vasotocin (VT) V_{1a}-like receptors modulates group size preferences in both sexes, and does so in a context-dependent manner that is dependent upon the subject's familiarity with the stimulus animals. A variety of data suggest that these context-dependent effects of V_{1a} antagonists reflect the opposing actions of VT neurons in the hypothalamus and extended amygdala, which are sensitive to stressors and positive social stimuli, respectively. Amydalar VT neurons are differentially activated by social stimuli in flocking versus territorial species, and both OT- and V_{1a}-like receptor densities reflect species differences in flocking behavior. Thus, across five estrildid finch species, species-typical group sizes correlate with nonapeptide receptor distributions in the lateral septum. These receptors appear to potently influence grouping, since sociality in female zebra finches is reduced by infusions of OT antagonist into the septum but not a control area. We propose that titration of sociality by MT represents a phylogenetically deep framework for the evolution of OT's female-specific roles in pair bonding and maternal functions, and further, that VT influenced social groupings in the earliest vertebrates, prior to the gene duplication that gave rise to separate OT and vasopressin lineages.

Molecular diversity of aquaporins closely associated with water adaptation strategy in anuran amphibians

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Anuran amphibians represent the first vertebrates that adapted to terrestrial environments, and are successfully distributed around the world, even to the forests and arid deserts. Many adult anurans have specialized osmoregulatory organs, in addition to the kidney, i.e. the ventral pelvic skin to absorb water from the external environments and a urinary bladder that stores water and reabsorbs it in times of need. Aquaporin (AQP), a water channel protein, plays a fundamental role in these water absorption/reabsorption processes. Anuran AQP family consists of at least AQP0-AQP5, AQP7- AQP10, and two anuran-specific types, designated as AQPa1 and AQPa2 (1). Basically, AQP3 is constitutively located in the basolateral membrane of the tight-junctioned epithelial cells of anuran osmoregulatory organs, allowing water transport between the cytoplasm of these cells and the neighbouring tissue fluid all time. On the other hand, different types of AQPs are expressed at the apical side of the tight epithelial cells: AQP2 in the kidney, h2-like AQPa2 in the urinary bladder, and h3-like AQPa2 in the ventral pelvic skin. All of them show translocation from the cytoplasmic pool to the apical plasma membrane in response to arginine vasotocin (AVT), thereby regulating water transport independently in each osmoregulatory organ. It was further revealed that, in terrestrial and arboreal anurans, the bladder-type AQP (h2-like AQPa2) is expressed in the pelvic skin, together with the pelvic skin-type AQP (h3-like AQPa2), potentially facilitating water absorption from the pelvic skin. In contrast, Xenopus has lost the ability of efficient protein production of the pelvic skin-type AQP because an extra C-terminal tail attenuates gene expression at a post-transcriptional step, which presumably leads to the prevention of the excessive water influx into this aquatic species. Collectively, the acquisition of two forms of AQPa2 and diversified regulation of their gene expression seem to provide the necessary mechanisms for the evolutionary adaptation of anurans to a wide variety of ecological environments.

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Vasopressin improves resuscitation outcome in out-of-hospital cardiac arrest
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Stress-induced behavior and plasma oxytocin levels during development are regulated dually by breast milk and hypothalamic ADP-ribosyl cyclase of CD38 in mice

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Influence of hormone treatment and mating conditions on anxiety-related behaviour and central oxytocin release in female rats

Martin Waldherr, Sandra Baeuml, Kewir Nyuyki, Oliver J. Bosch and Inga D. Neumann

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WCNH2009 Kitakyushu, JAPAN

Selected Communications

September 7th, 2009 16:15-17:45

VIIIth World Congress on

NeurohypophysialHormones

Kitakyushu, Japan

Program information

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Chaired by K Yamaguchi (Niigata, Japan) and A Douglas (Edinburgh, UK)

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P9 Phenylephrine (PE) induced increases in intracellular calcium ($[Ca^{++}]_i$) in supraoptic nucleus (SON) neurons are primarily mediated by a1A adrenergic receptors
Zhilin Song, Celia D. Sladek

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P12 Vasopressin and oxytocin can synaptically modulate hypoglossal motoneurons by activating excitatory and inhibitory premotor neurons
Ludovic Wrobel[1], Isabelle Reymond-Marron[2], Anouk Dupré[1], Eliane Tribollet[1], Mario Raggenbass[1]

16:45-17:00
P23 Neural correlates of abnormal aggression following peripubertal stress in rats: role of serotonin, vasopressin and oxytocin
Marquez Cristina, Vaucher Angélique, Sonnay Aliénor, Siegmund Coralie, Groner Anna Claire, Marquis Julien, Sandi Carmen

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P38 Glial control of synapse-specific actions of retrograde endocannabinoids in rat hypothalamic supraoptic nucleus
Shi Di[1], Jeffrey G. Tasker[1,2]

17:15-17:30
P59 Novel treatment for nephrogenic diabetes insipidus rat model using the sendai-virus vector carrying aquaporin 2 gene
Hidetaka Suga[1,2], Hiroshi Nagasaki[3], Taka-aki Kondo[1], Hiroshi Arima[1], Makoto Inoue[4], Mamoru Hasegawa[4], Yutaka Oiso[1]

17:30-17:45
P65 Aberrant intracellular calcium dynamics of neurohypophysis of thalidomide-induced autistic-like rats
Naoyuki Ishikita, Fumika Segawa

Poster Abstracts

P01

Vasopressin improves resuscitation outcome in out-of-hospital cardiac arrest

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Vasopressin is an attractive alternative to epinephrine during cardiopulmonary resuscitation (CPR), because it improves total cerebral and myocardial blood flow and causes a sustained increase in mean arterial blood pressure (MAP) compared to maximal doses of epinephrine. In cardiac arrest patients, endogenous vasopressin concentration increases within the first minute and is significantly higher in patients who are successfully resuscitated.
We conducted three different studies in prehospital setting [1-3] and came to conclusion, that the combination of vasopressine and epinephrine is more effective than epinephrine alone in the treatment of cardiac arrest.
Final end-tidal carbon dioxide values (Pet CO2) and average values of MAP in patients with restoration of pulse (ROSC) were significantly higher in vasopressin group ($p < 0.01$). Also significantly more ROSC and better 24 hours survival rates were observed ($p < 0.05$). Neurological outcome in discharged patients was better in the vasopressin group ($p = 0.04$).
In the second study we found in the subgroup of patients with myocardial infarction as the underlying cause of cardiac arrest, the hospital discharge rate was significantly higher in vasopressin treated patients ($p < 0.05$).
In the third study we investigated the influence of treatment with vasopressin and hydroxyethyl starch solution (HHS) on outcome in resuscitated blunt trauma patients with pulseless electrical activity (PEA). Higher average MAP and final Pet CO2 at admission were observed, followed by significantly higher ROSC [$p<0.01$] and better 24-hour survival rates [$p=0.001$] compared to standard treatment with epinephrine..
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P02

Roles for forebrain GABA receptors in the hypovolemia- induced vasopressin secretion in conscious rats

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Anteroventral third ventricular (AV3V) neural circuits that mediate vasopressin (AVP) secretion in response to the hypovolemia include the two subtypes of GABA receptor (-R), the GABA(A)- and GABA(B)-Rs. However, the problem of how those Rs may participate in the hypovolemic AVP secretion has not been elucidated as yet. This study aimed to pursue the issue through experiments in conscious and unrestrained rats with indwelling cerebral and vascular cannulae. Infusion sites in the brain were identified histologically after experiments. Topical AV3V infusion with bicuculline (Bic), a GABA(A)-R antagonist (Ant) caused marked rises in plasma AVP, osmolality (Osm), glucose, arterial pressure (AP) and heart rate (HR). These effects of Bic were blocked by preadministering with a GABA(A)-R agonist muscimol (Mus), the treatment alone did not exert appreciable action on the variables. The infusion with a GABA(B)-R Ant phaclofen affected none of the variables which we monitored. Removal of the systemic blood (1 ml/kg B.W.) through the arterial line did not change AP; neither raises nor alters plasma AVP and angiotensin (ANG) II. The same bleeding repeated after 10 min decreased AP, enhanced plasma Osm, and caused remarkable augmentations in both plasma AVP and ANG II. The responses of the AVP and ANG II were, respectively, blocked and potentiated by the AV3V infusion with Mus, whereas it did not affect the AP response. In contrast, AV3V infusion with a GABA(B)-R agonist baclofen (Bac) intensified the hemorrhagic AVP response, despite that the fall in AP was weakened, possibly due to tachycardia occurring concomitantly. Bac applied to the AV3V in the euvolemic state was verified to provoke pressor and tachycardiac actions and lack conspicuous effect on plasma AVP, Osm or electrolytes. These results suggest that under the hypovolemic circumstances GABA(A)- and GABA(B)-Rs in the AV3V may play opposing roles in controlling AVP secretion, different from the case played in the hyperosmotic state.

P03

Neurotransmitter regulation of c-*fos* and vasopressin gene expression in the rat supraoptic nucleus.

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Acute increases in plasma osmotic pressure produced by intraperitoneal injection of hypertonic NaCl are sensed by osmoreceptors in the brain, which excite the magnocellular neurons (MCNs) in the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) in the hypothalamus inducing the secretion of vasopressin (VP) into the general circulation (1). Such systemic osmotic stimulation also causes rapid and transient increases in the gene expression of c-fos and VP in the MCNs (2-6). In this study we evaluated potential signals that might be responsible for initiating these gene expression changes during acute hyperosmotic stimulation. We use an in vivo paradigm in which we stereotaxically deliver putative agonists and antagonists over the SON unilaterally, and use the contralateral SON in the same rat, exposed only to vehicle solutions, as the control SON. Quantitative real time-PCR was used to compare the levels of c-*fos* mRNA, and VP mRNA and VP heteronuclear (hn)RNA in the SON. We found that the ionotropic glutamate agonists (NMDA plus AMPA) caused an approximately 6-fold increase of c-*fos* gene expression in the SON, and some, but not all, G-coupled protein receptor agonists (e.g., phenylephrine, senktide, a NK-3-receptor agonist, and α-MSH) increased the c-*fos* gene expression in the SON from between 1.5 to 2-fold of the control SONs. However, none of these agonists were effective in increasing VP hnRNA as is seen with acute salt-loading. This indicates that the stimulus-transcription coupling mechanisms that underly the c-fos and VP transcription increases during acute osmotic

stimulation differ significantly from one another.

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P04

Down-regulation of oxytocin receptor mRNA in the medial amygdala plays a role in the formation of a long-term memory for a social hierarchy in rats

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Stress can have a major impact on social behaviour. We use a rat model to investigate the effect of a stressful experience on the establishment and maintenance of a hierarchy, a form of social behaviour that requires social recognition memory. Under our experimental conditions, rats that are exposed to a stressful experience before a first encounter with an unfamiliar unstressed rat typically become the submissive individual of the pair. Furthermore, the memory that is formed for the established hierarchy is enhanced by stress. Pairs of unstressed rats do not show a long-term memory for the established hierarchy.
We have previously shown a down-regulation in oxytocin receptor (OTR) mRNA in the medial amygdala (MeA) of the stressed subordinate rats 3h after the first encounter. We hypothesized that this down-regulation might underlie the enhancement of the memory for the hierarchy induced by stress. To test this hypothesis, an oxytocin receptor antagonist (OTA) was bilaterally injected into the MeA (1µg desGly-NH_2-d(CH_2)5[D-Tyr^2,Thr^4]OVT in 1?l of saline per side) of an unstressed subordinate rat ?that under normal conditions would not form a long-term memory for the hierarchy- either before or immediately after a first social encounter with another conspecific male rat. The effect of OTA treatments on the social memory was assessed in a water competition test given 1 week afterwards (and on the immediate social encounter when the antagonist was given before the first encounter).. Pairs of male Wistar rats were matched according to their anxiety level and body weight. Pairs were assigned randomly to three groups: (1) control pairs (both rats injected with saline either before or after the first encounter), (2) pairs in which the subordinate rat of the pair was injected with OTA and the dominant rat with saline immediately after the encounter and (3) pairs in which one rat was injected with OTA and one rat with saline 30 minutes before the encounter. Our results show that unstressed subordinate rats injected with OTA immediately after the encounter formed a long-term memory for the established hierarchy, in contrast to saline injected control rats. OTA injected before the first encounter had no long-term effect on the memory for the hierarchy.
The present results strongly indicate that the down-regulation of OTR plays a key role in the formation of a long-term memory for a social hierarchy.

P05

CCK effects in the SON and PVN are negatively modulated by leptin in 24h-fasted lean male rats

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Cholecytokinin (CCK) is an important satiety factor who regulates the amount of food consumed during an individual meal. In fed rats, peripheral injection of CCK activates neurones in the NTS, but also in several hypothalamic nuclei including the PVN, SON and DMH (1, 2, 3). Peripheral CCK still activates neurones in the PVN and NTS in fasted mice (4) but its effect on other hypothalamic nuclei or in fasted rats are less described. In a first experiment, we investigated whether peripheral CCK was increasing neuronal activity in the ARC, VMH, DMH and SON in fasted male rats by looking at the Fos expression in these nuclei. After being fasted for 24h, rats were injected ip with either vehicle (saline) or CCK-8 (15µg/rat). We found that in fasted rats, peripheral CCK increased Fos expression in the PVN and NTS but also in the SON. However CCK had no effect on Fos expression in the ARC, VMH and DMH seen in fasted rats.

Leptin, an anorectic hormone secreted mainly from adipocytes, is thought to play a role in the short-term regulation of feeding behaviour by interacting in synergy with CCK to reduce meal size. CCK has been shown to potentiate leptin effects in the PVN of fasted lean mice, suggesting that leptin may increase the efficacy of intra-meal satiety signals such as CCK (5). To clarify this hypothesis, we investigated whether peripheral leptin would modulate CCK effects on neuronal activity in the SON, PVN and NTS of fasted rats. Male rats, fasted for 24h, received either one ip injection of vehicle or leptin (50ug/ rat) alone, or received one injection of vehicle or leptin before an ip injection of CCK-8 (15µg/rat). As expected, we found that CCK increased Fos expression in the SON, PVN and NTS and that leptin injected alone had no effect. As previously reported in mice, we found that Fos expression in the PVN, SON and NTS of fasted rats injected with leptin and CCK was increased compared to the Fos expression seen in fasted rats injected with leptin alone. However the Fos expression in the PVN and SON of fasted rats injected with leptin and CCK was significantly reduced compared to fasted rats injected with CCK alone. Fos expression in the NTS was similar in fasted rats injected with CCK alone or with leptin and CCK.

Taken together, these results suggest that during fasting, leptin dampens the effects of CCK on Fos expression in the SON and PVN independently from NTS pathways and probably reflects a direct action on magnocellular neurones.

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P06

Changes in immediate early gene (Egr1) induction in vasopressin neurones in the anterior olfactory nucleus of the rat following social interactions

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Utilizing a transgenic rat that expresses an eGFP-vasopressin fusion gene (1), we detected previously undescribed populations of vasopressin neurones in the juxtaglomerular area of the main (MOB) and accessory olfactory bulbs (AOB), as well as within the anterior olfactory nucleus (AON). The AON is a central olfactory cortical structure with extensive connections to the olfactory bulb, and to the rest of the brain through the piriform cortex. As vasopressin has been established as an important neuroregulator of social behaviour, we quantified immediate early gene (Egr1) expression in vasopressin (eGFP) neurones in the MOB, AOB, and AON in lactating female rats following a maternal aggression test. During lactation, rats are exceptionally aggressive towards intruders and the neuroendocrine mechanisms controlling this behaviour require elucidation. We have previously shown that maternal aggression elicits immediate early gene induction in the rat olfactory bulb (2), but here we show that within the MOB and AOB very few of these activated cells are vasopressinergic and numbers of double labeled cells do not change following aggressive behaviour. Within the ventro-lateral extent of AON, the number of cells expressing both Egr1 and vasopressin was significantly lower in dams exposed to a maternal aggression test. The physiological relevance of this finding requires further research, but as experimental manipulation of vasopressin in the olfactory bulb modulates conspecific recognition (3), it is tempting to speculate that reduced vasopressinergic cell activity in this area of AON represents the coding of an olfactory memory of the intruder in the maternal aggression test.

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P07

Hunger and sexual motivation

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alpha -Melanocyte?Stimulating?Hormone (α-MSH) is a neuropeptide produced in neurones in the dorsomedial hypothalamus and in the arcuate nucleus of the hypothalamus. The central effects of α-MSH are similar to those of oxytocin: They both inhibit food intake and stimulate male sexual arousal (1, 2, 3). As feeding and sexual behaviours are mutually-exclusive goal-orientated behaviours, we investigated whether sexual motivation could overcome the drive for food in hungry rats and whether a-MSH and oxytocin were involved in the 'switch' of motivation from one drive to another.
We compared the sexual behaviour of hungry male rats to that of fed male rats. We found

that the percentage of males that mated did not differ between the fed and hungry rats but hungry males were slower to mate, reflected by significantly longer mount and intromission latencies. This suggests that in hungry rats, sexual motivation competes with the drive for food.
We also investigated whether administration of MC4R antagonist would mimic a hungry state and thus inhibit the sexual behaviour of fed rats. Administration of MC4R antagonist delayed all sexual behaviour parameters of fed rats to levels similar of those of hungry rats, and also reduced Fos expression (a marker of neuronal activation) in oxytocin neurones in the PVN, suggesting that some of the effects of α-MSH are mediated by oxytocin.
To better understand the regulation of sexual motivation in hungry rats we investigated the sexual incentive motivation (4) and looked at Fos expression in brain areas involved in sexual motivation. We found that like fed males, hungry males spent more time in the incentive zone of a receptive female compared to a non -receptive female and that Fos expression in the MPOA was significantly increased in hungry males when paired with a receptive female. In the PVN, Fos expression was significantly higher in hungry males than in fed males; however there was no additional effect of the sexual cues. Whether Fos expression in oxytocin neurones was altered is still under investigation.
Taken together these results suggest that while hungry rats can recognize sexual female cues and become sexually motivated, their sexual motivation does not overcome the drive for food. Thus the competition in motivations in hungry rats delays the transition to copulation. These results also indicate that α-MSH is involved, via MC4R, in the mechanisms regulating the switch in motivations, but whether oxytocin mediates α-MSH actions requires further investigations.

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P08

Oxytocin, given systemically, decreases methamphetamine self-administration, methamphetamine hyperactivity, and relapse to methamphetamine-seeking behaviour in rats

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Increasing evidence suggests the neurohypophysial nonapeptide oxytocin may be utilized as a treatment for various psychopathologies, including drug addictions. Here we used an animal model to assess whether oxytocin might be effective in the treatment of methamphetamine addiction. Sprague-Dawley rats were trained to lever press to intravenously self-administer methamphetamine under a progressive ratio schedule of reinforcement. Once responding had stabilized, one group of rats received escalating doses of oxytocin (0.001, 0.01, 0.1, 0.3, 1 mg/kg) administered intraperitoneally (IP) prior to daily self-administration tests while other rats received vehicle. After these tests, lever pressing was extinguished and the ability of methamphetamine primes (IP, 1 mg/kg) to reinstate responding was studied with and without co-administration of oxytocin (IP, 0.3 and 1 mg/kg). Results showed that oxytocin dose-dependently reduced responding for intravenous methamphetamine with an almost

complete absence of responding at the highest oxytocin dose (1 mg/kg). Hyperactivity during methamphetamine self-administration was also dose-dependently reduced by oxytocin. Oxytocin (1 but not 0.3 mg/kg) also reduced the ability of IP methamphetamine to reinstate methamphetamine seeking behavior. In separate tests, oxytocin (1 and 0.3 mg/kg) robustly decreased the hyperactivity induced by experimenter delivered methamphetamine (1 mg/kg, IP). This study suggests that oxytocin may have a powerful inhibitory effect on the motivation to consume methamphetamine. Ongoing research is exploring the neural basis of this effect. These results point to the possible utility of human trials of oxytocin as a therapeutic for methamphetamine addiction.

P09

Phenylephrine (PE) induced increases in intracellular calcium ([Ca^{++}]i) in supraoptic nucleus (SON) neurons are primarily mediated by a1A adrenergic receptors.

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Co-activation of a1-adrenergic receptors (a1-R) and purinergic 2 receptors by PE+ATP synergistically induces an increase in vasopressin release from the neurohypophysis (1) and an extended increase in [Ca^{++}]i in SON neurons (2). Three a1R subtypes are expressed in magnocellular neurons: a1A-R, a1B-R, and a1D-R, but their role in PE responses has not been assessed. A shift in the a1-R subtypes or recruitment of additional subtypes may underlie the synergistic response to PE+ATP. The current studies assessed the involvement of these three different a1-R subtypes in the PE-stimulated increase in [Ca^{++}]i in SON neurons. Hypothalamo-neurohypophyseal explants were loaded with fura-2 AM. Changes in [Ca^{++}]i were monitored by ratiometric analysis of fluorescence excited at 340 and 380 nm. PE induced increases in [Ca^{++}]i were completely suppressed by two specific a1A-R antagonists: WB4101 (100 nM) and 5-methylurapidil (1 mM); whereas neither the a1B-R specific antagonist AH11110A (100 nM) nor the a1D-R specific antagonist BMY7378 (10 and 100 nM) significantly affected the PE-induced [Ca^{++}]i increase. These results demonstrate that PE alone induced [Ca^{++}]i increase is primarily mediated through a1A-Rs. Further studies are on-going to identify the a1-R subtypes involved in the [Ca^{++}]i and hormone response to PE in combination with ATP. Supported by AHA SDG 0735329N and NIH R01 NS27975.

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P10

Effectiveness of nonpeptide vasopressin V_2 receptor antagonist tolvaptan in rats with heart failure

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Similar to other neurohormones that are activated in chronic heart failure (CHF), circulating arginine vasopressin (AVP) is elevated in patients with CHF. The precise role of AVP in the pathophysiology of cardiovascular disease is controversial. AVP is a peptide hormone that contributes to water retention and vasoconstriction in CHF through effects on V_2 and V_{1a} receptors, respectively. In the present study, the effect of V_2 receptor (V_2R) blockade using tolvaptan was assessed in a rat model of myosin-induced experimental autoimmune myocarditis. CHF was elicited in Lewis rats by immunization with porcine cardiac myosin, and 28 days after immunization rats were treated for 28 days with oral tolvaptan (3 or 10 mg/(kg day)) or vehicle. CHF was characterized by left ventricular remodeling and impaired systolic and diastolic function. Chronic V_2R blockade increased urine volume and urinary AVP excretion and decreased urine osmolality but had no natriuretic effect, and as a result caused increases in plasma osmolality and sodium. High doses of tolvaptan markedly elevated electrolyte-free water clearance. V_2R blockade did not activate the renin?angiotensin system, not influence cardiac remodeling, cardiac function, or survival. The upregulation of aquaporin 2 protein in the kidney of CHF rats was inhibited by the administration of V_2R antagonist. These results suggest that in a rat model of CHF, AVP may play a role in aggravating water retention through the renal V_2R.

P11

Electrophysiology study of vasopressin on rat olfactory bulb mitral cell activity

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Neural processing of olfactory signals is critical to social memory and appears to be dependent on the integrity of neuropeptides such as vasopressin (VP) and oxytocin. Previous study showed that infusion of VP directly into olfactory bulbs (OB) enhances social memory in rats (1), however, the role of endogenous VP in the OB is unknown.
In urethane-anaesthetised rats, we examined the effect of VP and a V1a receptor antagonist on OB mitral cells (MC) using extracellular recordings. The mitral cells were antidromically identified by electrical stimulation of the lateral olfactory tract, as reported previously (2). Most MC display conspicuous patterned discharge comprising prolonged intermittent bursts of action potentials. Many of these cells also display a bimodal interspike interval distribution reflecting the frequent occurrence of spike doublets. Topical administration of VP or the V1 receptor antagonist onto a small exposure of the OB significantly modified the electrical activity of these bimodal MC. VP reduced the activity quotient (proportion of time active), and particularly the doublet firing, whereas local application of a V1 antagonist had the opposite effect. Thus, VP seems to be a retrograde signal that filters activation of the MC arising from the glomeruli.

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P12

Vasopressin and oxytocin can synaptically modulate hypoglossal motoneurons by activating excitatory and inhibitory premotor neurons

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Hypoglossal (XII) motoneurons are located in the dorsal medulla of the brainstem and innervate tongue muscles. The nonapeptides vasopressin and oxytocin exert powerful excitatory effects on XII motoneurons. In addition, these peptides can enhance both inhibitory and excitatory synaptic inputs to XII motoneurons. The latter effect is probably due to a peptide-induced activation of neighboring premotor neurons. In the present work we attempted to localize and characterize at least part of these premotor neurons. We used brainstem slices of young rats, the patch clamp recording technique and immunohistochemistry. Recordings were obtained from neurons having the following properties. 1) They were located along the border of the XII nucleus, as confirmed by biocytin-labeling. 2) In dual labeling studies, biocytin-stained neurons were found to be cholinacetyltranferase (ChAT)-negative. 3) They could be antidromically activated following electrical stimulation from the XII nucleus. 4) Their action potential discharge pattern was characterized by the lack of a minimal discharge frequency. These properties indicate that these neurons were non-motoneurons and suggest that at least some of them may contact XII motoneurons. In the cell-attached configuration, we found that vasopressin and oxytocin could induce a reversible increase in the firing frequency in a majority of the recorded neurons. By using selective receptor agonists and antagonists, we assessed that vasopressin acted via V1a - but not V1b ? receptors, whereas oxytocin acted via uterine-type oxytocin receptors. The peptide effects were direct, since they persisted in conditions of synaptic blockade. We conclude that vasopressin- and oxytocin-responsive non-motoneuron cells located in the vicinity of the XII nucleus can mediate a peptide-dependent synaptic modulation of XII motoneurons. This mechanism may be of importance in motor pattern generation during feeding activity.

P13

Maternal aggression increases immediate early gene expression (c-*fos*) in oxytocin receptor expressing cells in the rat brain

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The dramatic rise in oxytocin and its receptor (OTR) just prior to parturition, due to the disinhibition of $GABA_A$ receptors, is essential for normal parturition and the milk ejection reflex during lactation. Such changes in the central oxytocin system during the peripartum period drive maternal behaviour including offspring protection from conspecifics termed “maternal aggression” (MA)[1,2]. The neuroendocrine circuits regulating this dramatic switch in behaviour require elucidation. Significant increases in OTR mRNA expression in the supraoptic nucleus, bed nucleus of stria terminalis (BnST) and medial preoptic area are linked with maternal be-

haviour implicating a regulatory role for oxytocin[1]. Here we investigated the hypothesis that the oxytocin system is involved in the display of aggression by quantifying c-*fos* protein expression in OTR labelled cells following a MA test. Female Sprague Dawley (SD) rats (Non-aggression tested (NAT) n=8, Aggression tested (AT) n=9) were mated and housed under standard laboratory conditions. Dams (lactation day 3-7 with their pups in their home cage) were subjected to a 30 min. MA test using a virgin female as an intruder or left undisturbed. Rats were perfused with 4% paraformalidehyde 90 min. after the start of the test and the brains collected and processed for double Fos and OTR immunocytochemistry. There was significant activation of OTR and Fos labelled cells in the BnST ($p < 0.001$; NAT=13.98± 3.7, AT=18.97± 2.7), medial amygdala ($p=0.001$; NAT=7.13± 1.1, AT= 18.82± 2.6) and lateral septum ($p < 0.001$; NAT=21.01±4.8, AT=46.21±12.8; an area already suggested to be specifically involved in maternal aggression $_3$). Together with previous findings showing oxytocin release in the amygdala during MA and that oxytocin antagonists block MA[2], it is clear that the oxytocin system plays a key role in regulating social behaviour such as female aggression.

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P14

Oxytocin mediates antidepressant effect by mating behavior and sildenafil in male mice

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Oxytocin (OXT) is known as a regulator of depression and anxiety behaviors. Moreover, recent studies have shown that OXT mediates mating-induced anxiolysis in male rats (1). Also, immunoassay of OXT, a neuropeptide hormone secreted by the posterior pituitary, indicated that sildenafil increased electrically evoked release (2). However, it is unclear whether OXT is involved in the antidepressant effect by mating behavior and sildenafil. In the present study, we examined the effect of mating behavior on depression behaviors in wild-type (WT) and OXT receptor-deficient (OXT-R KO) male mice. The depression behaviors were measured by forced swim test. Each animal was examined the duration of immobility during swimming for 6 min. In WT mice, the duration of immobility was significantly reduced at after termination of the mating behavior. In OXT-R KO mice, in contrast, the duration of immobility were not reduced compared with that before mating behavior. Sildenafil, i.p. injected 60 min after termination, significantly reduced the duration of immobility at doses of 60 mg/kg. In OXT-R KO mice, in contrast, the duration of immobility were not reduced compared with that before doses of 60 mg/kg. These results suggest that OXT is involved in the antidepressant effect by mating behavior and sildenafil in male mice.

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P15

Interleukin-6 Activates Arginine-Vasopressin Neurons in the Supraoptic Nucleus during Immune Challenge in Rats

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The increase of plasma Arginin-Vasopressin (AVP) release, which translates hypothalamic AVP neuron activation in response to immune challenge, appears to occur independently of plasma osmolality or blood pressure changes. Many studies have shown that major inflammatory mediators produced in response to peripheral inflammation, such as prostaglandin (PG)-E_2 and interleukin (IL)-1β, excite AVP neurons. However, *in vivo* electrical activation of AVP neurons was still not assessed in relation to plasma AVP release, osmolality, blood pressure or to the expression and role of inflammatory molecules, like PG-E_2, IL-1β, IL-6 and tumor necrosis factor (TNF)-a. This study aims at elucidating which factors underlie the activation of AVP neurons in response to immune stimulation mimicked by an intraperitoneal injection of lipopolysaccharide (LPS) in male Wistar rats. LPS treatment concomittanlty decreased diuresis and increased plasma AVP as well as AVP neuron activity *in vivo*, and these effects occurred as early as 30 min. Activation was sustained for more than six hours. Plasma osmolality did not change while blood pressure only transiently increased during the first hour post-LPS. PG-E_2, IL-1β and TNF-α mRNA expression raised 3 h after LPS, while IL-6 mRNA level increased 30 min post-LPS. *In vivo* electrophysiological recordings showed that brain IL-6 injection increased AVP neuron activity similarly to peripheral LPS treatment. In contrast, brain injection of anti-IL-6 antibodies prevented the LPS induced-activation of AVP neurons. Taken together, these results suggest that the early activation of AVP neurons in response to LPS injection is induced by brain IL-6.

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P16

Specific expression of the oxytocin-enhanced cyan fluorescent protein fusion transgene in the rat hypothalamus and posterior pituitary

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In order to facilitate that facile visualization of oxytocin (OXT)-producing neurons in the hypothalamus and their terminals in the posterior pituitary (PP), we have generated rats bearing the OXT-enhanced cyan fluorescent protein (eCFP) fusion transgene designed from a murine construct previously shown to be faithfully expressed in transgenic mice. In situ hybridization histochemistry revealed that the OXT-eCFP fusion gene was expressed in the supraoptic (SON) and the paraventricular nuclei (PVN) in these rats. The fluorescence emanating from eCFP was observed only in the SON, the PVN, the internal layer of the median eminence (ME) and the PP. Immunohistochemistry for OXT and arginine vasopressin (AVP) revealed that the eCFP fluorescence co-localizes with OXT-immunofluorescence, but not with AVP-immunofluorescence in the SON and the PVN. Although the expression levels of the OXT-eCFP fusion gene in the SON and the PVN showed a wide range of variation in euhydrated and salt loaded male and female transgenic rats, eCFP fluorescence was markedly increased in the SON and the PVN, but decreased in the PP after chronic salt loading. The expression of the OXT gene was significantly increased in the SON and the PVN after chronic salt loading in both non-transgenic and transgenic rats. Compared to wild-type animals, euhydrated and salt-loaded male and female transgenic rats showed no significant differences in plasma osmolality, sodium concentration, OXT and AVP levels, suggesting that the fusion gene expression did not disturb any physiological processes. In in vitro preparations, freshly dissociated cells from the SON and axon terminals showed clear eCFP fluorescence. Taken together, these results suggest that our new transgenic rat is a valuable new tool with which to identify and evaluate the physiological regulation and functions aspects of OXT-producing neurons and their terminals.

P17

Immunohistochemical distribution of water channel, AQP4, in the rat central nervous system

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Aquaporins are membrane water channel proteins through which water as well as some solutes permeates lipid bilayer. So far thirteen aquaporin isoforms, namely aquaporin 0 (AQP0) to AQP12, have been identified. In the central nervous system, AQP4 is expressed. It is known that AQP4 is highly concentrated in the perivascular end-feet as well as ependymal epithelium. It could play an important role in water transfer between the blood vessels or ventricle and brain parenchyma. In the present study we raised the specific antibody against AQP4 and surveyed the distribution of AQP4 in the rat central nervous system. AQP4 was diffusely distributed throughout the central nervous system. Double-labeling for AQP4 and glucose transporter 1 as a blood vessel marker showed that AQP4-distribution was not restricted to the perivascular end-feet. Judging from fiber-or fibril-like labeling pattern as well as the results of double-labeling for AQP4 and glial fibrillary acidic protein as an astrocyte marker, AQP4 seems to be broadly distributed in astrosytes. In addition to the perivascular end-feet, AQP4-labeling was highly concentrated in some areas such as: 1) supraoptic nucleus, nucleus of trapezoid body, as well as glomerular layer of the olfactory bulb, where neurons were surrounded by strong labeling for AQP4, and 2) medial geniculate nucleus, where

lots of small circle-like structures were strongly labeled. Immunoelectron microscopy we are performing now would give further helpful information. Our results suggest that astrocytes play important roles in water handling in the central nervous system.

P18

Deficiency of vasopressin V_{1A} and V_{1B} receptors results in impaired glucose tolerance

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[Arg^8]-vasopressin (AVP) is involved in the regulation of glucose homeostasis via vasopressin V_{1A} and vasopressin V_{1B} receptors. Our previous studies have demonstrated that vasopressin V_{1A} receptor deficient (V_{1A}R-/-) mice exhibit hyperglycemia (1), while vasopressin V_{1B} receptor deficient (V_{1B}R-/-) mice, in contrast, exhibit hypoglycemia with hypoinsulinemia (2). These findings indicate that vasopressin V_{1A} receptor deficiency results in decreased insulin sensitivity, whereas vasopressin V_{1B} receptor deficiency results in increased insulin sensitivity. In the present studies, in order to assess the effect of deficiency in vasopressin V1B receptor on the development of glucose intolerance caused by vasopressin V_{1A} receptor deficiency, we generated mice that were deficient for both vasopressin V_{1A} receptor and vasopressin V_{1B} receptor (V_{1AB}R-/-) and examined their glucose tolerances. The glucose tolerance test demonstrated that glucose tolerance was impaired in V_{1AB}R-/- mice, suggesting that the effects of vasopressin V_{1B} receptor deficiency could not influence the development of hyperglycemia promoted by vasopressin V_{1A} receptor deficiency, and that blockade of both receptors could lead to impaired glucose tolerance. Hyperglycemia with insulin resistance observed in V_{1A}R-/- mice is at least in part due to decreased plasma volume, increased glycogenolysis, and/or decreased insulin signal in the adipocytes (1). Hypoglycemia with hypoinsulinemia observed in V_{1B}R-/- mice is probably due to enhanced insulin signal in the adipocytes (2). Therefore, we examined the insulin signal in the adipocytes of V_{1AB}R-/- mice. The phosphorylation of Akt in V_{1AB}R-/- mice was decreased compared to that in WT mice as well as V_{1A}R-/- mice, indicating that the insulin signal was suppressed in the adipocytes of V_{1AB}R-/- mice. These results suggested that the impaired insulin signal caused by vasopressin V_{1A} receptor deficiency could not be affected by the effects of vasopressin V_{1B} receptor deficiency and that the impairment of glucose tolerance in V_{1AB}R-/- mice could result from the impaired insulin signal.

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P19

Oxytocin Facilitates Female Sexual Maturation in Mouse

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Oxytocin (OXT) and oxytocin receptor (OXTR) play important roles in a wide spectrum of central and peripheral system. The recent finding demonstrated that administration of oxytocin antagonist blunted the preovulatory LH peak in women and that of an oxytocin antagonist to female rat showed delay in their sexual maturation, which involved a decrease on frequency of pulsatile GnRH secretion. A number of experiments showed that oxytocin might regulate the GnRH cells, suggesting a possible modulation on LH surge. OXTR have been found in several brain regions, including medial preoptic area (MPOA), where also the GnRH neurons are distributed in rats, and recently we also detected possible expression of OXTR in mouse MPOA, by generating OXTR-Venus knock-in mice (1). In mice it is not known whether GnRH neurons themselves express Oxtr. Here we observed vaginal opening and vaginal lavages in mice lacking OXT *(Oxt-/-)*. Both vaginal opening and the age of the onset of the first estrus were significantly delayed in Oxt-/- female mice. Next we studied the presence of Oxtr in GnRH cells of the MPOA using Oxtr-Venus knockin mice.

The effect of some Prostanoid (PG) mimicked the stimulatory effect of Oxt on GnRH pulse frequency, and inhibition of Prostaglandin E2 synthesis blocked the effect of Oxt. We found disturbed estrus cycle in mice lacking OXT and PGF2a receptor *(Oxt-/-, Fp-/-)*. These results strongly suggest that Oxt facilitates female sexual development and maturation, and this effect is suspected to be mediated by PG production and/or GnRH secretion, at least partially.

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P20

Varying Distribution of Serum Sodium Closely Associated with Enhanced Secretion of Vasopressin in Subjects with Acute Infectious Diseases

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The present study was undertaken to determine association of serum sodium (Na) and circulating blood volume with arginine vasopressin (AVP) release in subjects with acute infectious diseases. A total of 63 subjects were enrolled in the present study. They were 31 males and 32 females, with the ages of 74.9 ± 13.5 years (mean ± SEM) ranging from 32 to 96 years. Serum Na, plasma AVP and interleukin (IL)-1β were determined at the hospitalization, 48 hours after the hospitalization and at the discharge. The subjects were subgrouped into 3 groups; Group A: serum Na $\leqq$ 134 mmol/l, Group B: 134 $<$ serum Na $\leqq$ 140 mmol/l, and Group C: serum Na $>$ 140 mmol/l. Group A of hyponatremic subjects had serum Na levels of 124.7 mmol/l, whose % change in circulatory blood volume (CBV) was +1.8% as compared to the discharge. Group B of the normo-natremic subjects and Group C of the supernormonatremic subjects had serum Na levels were 135.8 and 145.0 mmol/l, and their % change in CBV was -4.7 and -6.0%, respectively. Plasma AVP levels were extremely as high as more than 10 pg/ml in all the 3 groups. Plasma IL-1β was also elevated in all the 3 groups, especially 13.0 pg/ml in the hyponatremic subjects. After the hospitalization, antibiotics and fluid

infusion therapy had been carried out. 48 hours after the hospitalization, % change in CBV returned to approximately 0% in the hypo- and normonatremic groups, and plasma AVP levels were significantly reduced but still remained elevated. Plasma IL-1β remained high in only the hyponatremic subjects. Plasma AVP levels were inappropriately increased by IL-1β in the hyponatremic subjects, who kept circulatory blood volume normal. In contrast, elevated plasma AVP levels were closely associated with circulatory blood volume depletion in the normo- and supernormo-natremic subjects. These findings indicate that there are two patterns of appropriate and inappropriate secretion of AVP in concert with circulatory blood volume state and inflammatory cytokines in acute infectious diseases. Particularly, hyponatremia is closely linked to inflammatory cytokine-induced inappropriate secretion of AVP.

P21

Activation of CD38 involved in autoregulation of oxytocin secretion in the hypothalamus and posterior pituitary in male mice

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Oxytocin induces its own release from oxytocinergic neurons in the hypothalamus is a well-known positive feedback loop in labor, as well as lactation (1). Although oxytocin mobilizes Ca^{2+} by IP3 from thapsigargin-sensitive intracellular store has been previously described (2), but based on our study of the CD38-mediated oxytocin secretion in hypothalamus and posterior pituitary (3), it is interesting to further investigate whether CD38-cADPR-[Ca^{2+}]i signaling is also involved in oxytocin-induced oxytocin release or not. We found that protein kinase C which was generated from oxytocin-receptor coupling G-protein pathway and it could promote the ADP-ribosyl cyclase activity of CD38, leading to produce more cADPR, a potential second messenger to modulate intracellular calcium increase from endoplasmic reticulum and further enhance oxytocin release. The above effects in response to oxytocin stimulation were largely inhibited by PKC inhibitor. Therefore, our results demonstrate the existence of a signal pathway from oxytocin receptor to membrane-bound ADP-ribosyl cyclase of CD38 via $G_{q/11}$ protein in the hypothalamus and neurohypophsis and suggest that cADPR-mediated intracellular calcium increase is involved in auto-regulation of oxytocin release.

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P22

Expression of (pro)renin receptor in the human hypothalamus and co-localization with arginine vasopression in the paraventricular magnocellular neurons

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(Pro)renin receptor ((P)RR), a specific receptor for renin and prorenin, is a 350 amino-acid protein with a single transmembrane domain and widely expressed in various tissues including brain, heart and kidney. When bound to (pro)renin, (P)RR activates the angiotensin I-generating activity of (pro)renin in the absence of cleavage of the prosegment, and directly stimulates the MAPK pathway independently from the renin-angiotensin-aldosterone system (RAS). In the present study, expression of (pro)renin receptor and co-localization with arginine vasopression in the human hypothalamus were studied by immunocytochemistry. The present study was approved by the Ethics Committee of the Tohoku University Graduate School of Medicine. Human hypothalami were obtained at autopsy from the subjects without neurological or endocrinological disorders. Immunocytochemistry was performed by the ABC method using paraffin-embedded sections. The antiserum against (P)RR was raised in a rabbit by injecting the peptide fragment of human (P)RR corresponding to 224-237 a.a. conjugated with bovine serum albumin. The identity of the (P)RR immunoreactivity was confirmed by Western blot analysis, which showed a band of 39 kDa in the protein extract of rat kidney and heart. Furthermore, the preabsorption of the antibody by the antigen peptide abolished the immunostaining of (P)RR in immunocytochemistry of the human hypothalamus. Immunocytochemistry showed that (P)RR was expressed in the paraventricular and supraoptic nuclei of human hypothalami. Immunostaining of serial sections showed that (P)RR was co-localized with arginine vasopression in the magnocellular neurons of the paraventricular nucleus. The present study has shown that (P)RR is expressed in human hypothalamus. These findings have raised the possibility that (P)RR may activate the central RAS, and be related to the central control of electrolyte-water metabolism, blood pressure regulation and vasopression release.

P23

Neural correlates of abnormal aggression following peripubertal stress in rats: role of serotonin, vasopressin and oxytocin.

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Early life stress in humans enhances the risk for psychopathologies, including excessive aggression and violence. Adolescence is an important maturation phase during which critical neurodevelopmental events occur in brain regions associated with motivation, emotion and cognition. It is also a relevant period for developing social competences required for adult life. In rodents, stress can induce long-lasting changes in emotional and neuroendocrine responsiveness to stress that can be associated with several psychopathologies. Serotonin, vasopressin (AVP) and oxytocin (OXT) have been proposed as important mediators of the pathogenesis of these stress-related disorders, and have been proposed as important modulators of social behavior. In the last years our laboratory has developed a new animal model of abnormal aggression, focused on the consequences of exposure to stress during the peripubertal period. Male Wistar rats were subchronically exposed to stress (predator odour and open elevated spaces) during peripuberty (7 days of stress across the P28-P42 period).

The long term effects of peripubertal stress were examined when animals were 3 months-old. Peripuberty-stressed animals displayed increased anxiety and aggressive behaviors. In the resident-intruder test, peripuberty-stressed rats attacked more the vulnerable parts of their opponent and showed a lack of inhibitory control of their behavior (continued attacking despite clear signals of submission). In order to elucidate the mechanisms involved in this model of abnormal aggression, we studied (i) basal brain energy metabolism using ^{14}C-2de-oxyglucose autoradiography, (ii) the pattern of activation of different brain areas after an aggressive encounter (resident-intruder test) using c-fos immunohistochemistry, (iii) the levels of expression of the serotonin transporter, AVP 1a receptor and OXT receptor using qPCR. Peripuberty-stressed animals showed an increased basal metabolism in amygdala and bed nucleus of stria terminalis, both areas related to anxiety, and an increase in the basal expression of the serotonin transporter in the prefrontal cortex. They showed increase in c-fos expression in medial and central amygdala and a lower activation of the medial orbitofrontal cortex, after the resident-intruder test. Our findings highlight the relevance of this peripubertal stress model to investigate the neurobiological correlates of abnormal aggression and confirm the serotonergic, AVP and OXT systems and the interactions between amygdala and prefrontal cortex as key elements in the understanding of violence.

P24

Injection of AAV-Oxtr viral vector into brain of OXTR-KO mice recovered maternal behavior

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The oxytocin receptor (OXTR) and its ligand, oxytocin (OXT), exert multiple effects in regulations of reproductive function and sociosexual behaviors. Oxtr knock-out mice (Oxtr-/-) exhibited normal parturition but defect in lactation and maternal behaviors (1). In the test of maternal behaviors, impairment in retrieving of infants was observed with the Oxtr-/-, but not with wild-type mice. This dampened maternal behavior was observed only in the mice lacking Oxtr gene, but was irrelevant to the mice lacking the Oxt gene. Lateral septum(LS) of the brain was reported to be one of the centers regulating maternal behaviors, and we detected the expression of OXTR in this area using Oxtr-Venus mice, which we recently generated (2). In addition, immunohistochemistry of the LS nucleus prepared from Oxtr-/- mice showed decrease in the c-fos expression, which was generally considered as a marker of neuronal activation, in comparison with a wild type.
We developed AAV vector, which could introduce Oxtr gene to the neuronal cells with Venus, the fluorescence marker gene (3). The rescue experiment for the maternal behavior, using Oxtr-AAV vector injection into maternal behavior-related areas in brain, was carried out. In LS nucleus of the wild type mice (Oxtr(+/venus)), we confirmed the expression of Oxtr (2). The resultant Oxtr-/- mice showed an increased phenotype in the maternal behavior. In the LS nucleuses of the vector-injected mice, it was confirmed to express Venus maker after the behavioral test.
This experiment strongly suggested that the expression of OXTR in the LS nucleus had a critical effect on the recovery of maternal behaviors in Oxtr-/- mice. We continuously study the effect of the Oxtr expression in various regions in mice brain, including LS, MPOA, MnR, DR and so on, on the maternal behaviors.

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P25

Parenting or infanticide: context-dependent behavioral choice and region-specific neuronal activation in male mice.

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For all mammalian infants, parental care is essential for survival. In rodents they typically display a stereotyped set of parental behaviors (e.g., retrieving, licking, nest building, nursing). In laboratory mice, fathers also care for their offspring like mothers. But when these males are virgin, 50~90% of them (substrain differences) commit infanticide (pup-killing). The infanticide of non-progeny young by males is an adaptive behavior in many mammals to increase their reproductive success. Such infanticide by virgin male mice are inhibited after copulation and cohabitation with the pregnant females by 19 days, which is the time the pups would be born, and most males become parental toward pups (Vom Saal, 1978; Parmigiani, 1991). Thus copulatory stimuli and cohabitation with a pregnant female appear to inhibit infanticide and facilitate parenting toward pups. It is not clear, however, where and how the memory of such a social experience with the female is stored. To elucidate this experience-dependent behavioral change mechanism, we examined the brain regions activated by parenting, infanticide, or copulation using immediate-early gene c-Fos immunoreactivity.
As a result we identified some different brain regions activated by parenting or infanticide, respectively. Especially, c-Fos immunoreactivities in the accessory olfactory bulb, extended amygdala, and the hypothalamus showed different expression patterns induced by the same pup stimuli in infanticidal and parental males.

P26

Effects of i.c.v. administration of RFamide related peptide-1 upon release of stress-related hormones and anxiety-related behavior

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RFamide related peptide-1 (RFRP-1) is a neuropeptide that contains an arginine-phenylalanine amide structure at its carboxy-terminal. RFRP-1-expressing neurons are found to be localized in the hypothalamic paraventricular nucleus and dorsomedial hypothalamus. These nuclei play an important role in the control of stress responses. However, the role of RFRP-1 in the control of stress responses remains unknown. Here, we examined effects of i.c.v. administration of RFRP-1 upon plasma concentrations of vasopressin, oxytocin and ACTH, and upon anxiety-related behavior in male Wistar rats. Administration of RFRP-1 increased plasma oxytocin and ACTH but not vasopressin concentrations. After i.c.v. administration of RFRP-1, animals showed anxiety-related behaviors in an open field test. Psychologically stressful stimuli have been shown to facilitate oxytocin and ACTH but not vasopressin release from the pituitary, and to induce anxiety-related behavior. All these data are consistent with a

view that RFRP-1 plays a role in the control of neuroendocrine and behavioral responses to stress.

P27

Localization, chemical identity and connectivity of oxytocin-receptor expressing neurons in the mouse spinal cord

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Substantial evidence obtained mostly in the rat suggests that oxytocin (OT) is involved in a variety of spinal functions. Thus, a dense OT innervation, originating from the hypothalamic paraventricular nucleus, has been described at different spinal segmental levels. High affinity binding sites have also been detected in different spinal areas, although in low density in adult animals. Functional studies suggest that OT exerts antinociception and analgesia by acting on neurons localized in the superficial layers of the dorsal horn, may contribute to the regulation of the autonomic nervous system by influencing both preganglionic sympathetic and parasympathetic neurons, and facilitates penile erection when administered intrathecally at lumbosacral spinal levels. Little is known however about the neuronal spinal networks influenced by OT, this being due mainly to the difficulty to identify neurons which express OTRs. Indeed, in vitro autoradiography does not allow the localization of binding sites at the cellular level, and anti-OTR antibodies available so far are not reliable. In the present study, we have used a knock-in mouse, in which the fluorescent protein Venus is expressed under the control of the endogeneous OTR gene promoter (1) to study OTR expressing neurons. Our aim was to 1) localize and map OTR expressing neurons at all segmental levels; 2) investigate their chemical identity; and 3) assess whether they receive OT-containing afferent axons. To this end we have used an anti-GFP antibody to detect Venus-expressing neurons in combination with one of the following antibodies: anti OT-neurophysin, anti-NeuN, anti-ChAT, anti-substance P or anti-GAD. Results obtained so far show that Venus immunoreactivity is widely distributed in the mouse spinal cord: in about 30% of neurons in lamina II of the dorsal horn, in autonomic regions where it is co-localized with ChAT immunoreactivity in some, but not all, neurons, and also in numerous neurons scattered in different regions of the central gray matter at all levels. Data obtained with other antibodies are currently analyzed. Altogether, our results show that the OTR-Venus knock-in mice offer a powerful new tool to investigate the possible functions of OT in the spinal cord.

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P28

Circadian rhythm of vasopressin-enhanced green fluorescent protein fusion gene expression in the rat suprachiasmatic nucleus

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We have recently developed a new transgenic rat expressing arginine vasopressin (AVP)-enhanced green fluorescent protein (eGFP) fusion gene and have demonstrated the value of this animal model in understanding the physiology of AVP neurons of the central nervous system (CNS). In the present study we examined daily profiles of the expression of the eGFP, AVP and period (Per1, Per2) genes in AVP-eGFP transgenic rats that express eGFP in hypothalamic AVP-containing neurons. The gene expression in the suprachiasmatic nucleus (SCN) of the AVP-eGFP transgenic rats showed a circadian rhythm. These daily profiles were similar to those expressed in non-transgenic rats. *In situ* hybridization histochemistry revealed that the rate of change in the rhythm of eGFP gene expression was significantly greater than that of the AVP gene. We also examined the effect of light stimulus on the expression of each gene (AVP, per1 and per2) in the SCN of the transgenic rats. Ninety minutes after light stimulus, eGFP mRNA and AVP hnRNA levels in the SCN were significantly decreased while Per2 mRNA levels were significantly increased. Our results indicate that the introduction of the foreign gene in the AVP-eGFP transgenic rats has no influence on the intrinsic circadian rhythm. It is therefore possible to use the eGFP gene to examine the expression of the AVP gene in the SCN. The AVP-eGFP transgenic rat is an interesting animal model to study the circadian rhythm and dynamics of the AVP system *in vivo*.

P29

Epithelial sodium channels in the vasopressin and oxytocin synthesizing neurons of the rat hypothalamic supraoptic nucleus

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The non-voltage-dependent, amiloride-sensitive Epithelial Na^+ channels (ENaCs) are present in kidney and are known to contribute to Na^+ and water homeostasis. All three ENaC subunits (α, β, and γ) have also been demonstrated in the cardiovascular regulatory centers of the rat brain including the magnocellular neurons (MNCs) in the supraoptic nucleus (SON) and the paraventricular nucleus. In addition, a known target for ENaC expression, the mineralcorticoid receptor (MR), is present in MNCs. In humans, most of the known genetic causes of hypertension are due to defects in ENaC itself or its regulation, which results in abnormal increases in renal Na^+ reabsorption. Intracerebroventricular (ICV) injections of the ENaC blocker, the amiloride analogue benzamil, significantly attenuated the hypertension in animal models with salt-dependent forms of hypertension. Despite these findings, the functional significance of ENaCs in MNCs is completely unknown. In the present study, we looked for evidence of an amiloride-sensitive current in MNCs using whole voltage-clamp in the SON in rat brain slices. Steady state current and input conductance were monitored while the MNC was held at -70 mV. A bath application of amiloride reversibly reduced the steady state inward currents and

decreased cell membrane conductance by approximately 2-fold. In addition, we successfully detected mRNA for all three ENaC subunits (α, β, and γ) and MR in a cDNA library derived from single MNCs by RT-PCR. Finally, all ENaC subunits and MR immunoreacitivities were co-localized with OT-neurophysin (OT-NP) or VP-NP immunoreactivities in the SON using confocal microscope. These results strongly suggest the presence of functional ENaCs in MNCs that contribute to an inward leak current. Thus, ENaCs may affect the firing patterns of MNCs, which ultimately control the secretion of these hormones, either by changes in membrane potential, and/or by altering the balance of Na^+ in and outside the neuron.

P30

TonEBP regulates hyperosmolality-induced arginine vasotocin gene expression in the chick hypothalamus (*Gallus domesticus*)

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Arginine vasotocin (AVT) is expressed mainly in the paraventircular and supraoptic nuclei of the hypothalamus in chicken. AVT is known to act as an antidiuretic hormone and its gene expression is stimulated by hyperosmolality. However, the transcription factors that actually regulate the hyperosmolality-induced AVT gene expression are still unknown. In this study, we examined the role of hyper-tonicity enhancer binding protein (TonEBP) in the transcriptional regulation of the AVT gene in chicken. We found that TonEBP mRNA expression levels increased at 1 hr after salt-loading treatment in the hypothalamus. This increase preceded that of AVT and c-fos mRNA expression. Intracerebroventricular injections of TonEBP antisense oligonucleotide before the salt-loading treatment inhibited the increase in AVT gene expression. These results, all together, suggest that the transcription factor TonEBP may be involved in the regulation of AVT genes expression in response of hyperosmotic environment in chicken.

P31

Modulation of hypothalamopituitary axis in Mini rat: further studies with hypophysiotropic somatostatin and gonadotroph

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Mini rats are transgenic animals carrying an antisense RNA transgene for rat growth hormone (GH); they show dwarf phenotype and a low blood GH level compared to age-matched wild-type Wistar (non-Mini) rats at 4, 6 and 8 weeks of age. Although the effects of GH on normal development and body growth have been clearly established, the action on gonad via the hypothalamus-pituitary (H-P) axis has been poorly understood. Previously, reported that

the GH-immunoreactive (IR) cell and anterior pituitary (AP) size of Mini rats were significantly lower than that the non-Mini rats (1).
The purpose was to investigate the parameters of somatostatin (SS)-IR cell and Lutenizing Hormone (LH)-IR cell, to address these questions by applying immunohistochemical and morphmetric methods to the hypothalamus and pituitary gland. As a result, the parameter of LH-IR cell in Mini rats was significantly higher than non-Mini rats at 4 weeks of age. SS-IR cell in non-Mini rats was gradual decrease with 4, 6 and 8 weeks of age, whereas in Mini rats it seemed to remain strong at respective group. In the spermatogenesis was detected the acrosomal formation, whereas unsynchronized in Mini rat.
These results suggest that the abnormal population of SS-IR cells in the hypothalamus was modulated by the GH antisense RNA transgene, it was also responsible for the somatic growth and gonadal function. Moreover, parameters of LH cell results showed that intrinsic GH might directly modulate the peripheral cell in the pituitary, as well as orchestrate by hypophysiotropic SS project into infundibulum from the hypothalamic nucleuses. Our results indicated that the Mini rat was unique for hypothalamo-neurohypophysial research.

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P32

Impaired thermoregulatory ability in mice lacking oxytocin gene and oxytocin receptor gene during cold-exposure

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Oxytocin (OXT) and its receptor (OXTR) play important roles in reproductive functions, social behaviors and so on.
We first found that OXT deficient (*Oxt*$^{-/-}$) mice had deficits in maintaining their body temperature during exposure of them to a cold environment (1), and OXTR deficient (*Oxtr* $^{-/-}$) mice also failed to maintain their body temperature after acute cold exposure (2). Histological sections of brown adipose tissue (BAT), which generates body heat to control the body temperature, from *Oxtr* $^{-/-}$ mice showed a number of adipocytes filled with large lipid droplets and a marked decrease in multilocular adipocytes, a typical feature of functionally inactive BAT (2). In the BAT of male *Oxtr* $^{-/-}$ mice, the expression of uncoupling protein 1 (UCP1) normally increased by cold exposure, but the mRNA level of β3 adrenergic receptor (AR) were lower than that of wild type mice at room temperature (RT) and mRNA level of α2 AR were higher both at RT and at cold temperature. Since these ARs were known to have opposite effects on the thermoregulation, the imbalance of ARs might cause this dysfunction in the thermoregulation.
We predicted that OXT/OXTR systems controlled the thermoregulation via central nervous system because both OXT and OXTR were mainly expressed in the brain but not in mature brown adipocyte. Double immunofluorescent staining of the paraventricular nucleus (PVN) in the wild type brain showed that several OXT neurons co-expressed c-Fos immunoreactivity

when the mice were exposed to the cold environment (1).

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P33

Mammillary body-upstream center controlling synchronization of milk-ejection bursts in rat bilateral supraoptic oxytocin neurons

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Successful milk ejections depend on activation of hypothalamic oxytocin (OT) neurons in burst discharges in a synchronized manner. Without suckling stimulation, burst-like firing can still be detected in OT neurons in vivo and in vitro but synchronization was not observed. Thus, it is likely that the synchrony in OT neurons is controlled by exogenous synchronizing signals generated on some loci linking to the afferent pathway of suckling signals. The integrative cell groups for bilateral OT burst synchrony have remained enigmatic. Cooperative research among several laboratories has finally culminated in a set of results defining the brain region causally involved in producing the synchronous bursting of OT neurons. Using microsurgery and paired recordings from bilateral supraoptic OT neurons, we identified that disconnections of bilateral sides of the mammillary body resulted in burst de-synchronization but did not block burst generation. Moreover, lesion or blockage of this region with neurotoxins led to starvation of pups while disturbing the rhythmic activity of burst firing. In this region, firing activity of many tuberomammillary neurons precipitously declined while a few neurons in this region discharge burst-like firing before each milk ejection, time-locked to the milk-ejection bursts. Morphologically, reciprocal projections were found between histamine receptor-bearing OT neurons and OT receptor-bearing histaminergic neurons. One histamine neuron can innervate OT neurons in bilateral SON simultaneously while histamine neurons mutually communicated via gap junctions. Functionally, OT suppressed tuberomammillary neuronal activity recorded in brain slices while eliciting burst firing in a few of them; histamine, similarly, inhibited OT neuronal activity while triggering burst in a portion of OT neurons. From these results, it is concluded that a synchronization “center” for the bursts of bilateral OT neurons is located in the region of mammillary nuclei, and tuberomammillary histaminergic neurons are the *sine qua non* for the bilateral synchronization of bursts.
This work is supported by grants from Monbukagakusho, Japan, NIH (NS009140), USA, and Jiamusi University, China. *, present address: Yu-Feng Wang, Department of Cellular Biology & Neuroscience, LSUHSC-Shreveport, LA 71130, USA.

P34

Role of oxytocin receptors in the control of stress-induced energy expenditure

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The oxytocin receptor has been suggested to be involved in energy metabolism, such as food intake and energy consumption. We found that oxytocin receptor-deficient male mice show late-onset obesity (1). Daily food intake and spontaneous motor activity of oxytocin receptor-deficient male mice were not significantly different as compared with wild-type mice. On the other hand, brown adipose tissue in oxytocin receptor-deficient male mice contained large lipid droplets and cold-induced thermogenesis was impaired in oxytocin receptor-deficient mice (1). These results suggest that the development of obesity in oxytocin receptor-deficient mice may be due to a decrease in energy expenditure. In the present study, we examined oxygen consumption in oxytocin receptor-deficient male mice under basal condition or cage-switch stress. Basal oxygen consumption was not significantly different between oxytocin receptor-deficient and wild-type animals. Cage-switch stress increased oxygen consumption in both wild-type and oxytocin receptor-deficient mice. However, the increase was significantly lower in oxytocin receptor-deficient mice. These results suggest that the oxytocin receptor plays an important role in stress-induced energy expenditure and that a reduced increase in oxygen consumption induced by cage-switch stress might have contributed to the development of obesity in oxytocin receptor-deficient mice.

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P35

Possible role for anterior commissure nucleus in the regulation of mouse maternal behaviors

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Maternal care of the young is a common feature among mammals, so that the basic neural circuit for maternal behavior should be conserved among mammals. Laboratory rats and mice have been major model organisms for maternal behavior study. In these rodents, several findings suggest that the dorsolateral part of the medial preoptic area (MPOAdl) in the hypothalamus and the adjacent ventral part of the bed nucleus of stria terminalis (BSTv) are critically involved in the neural control of the maternal behavior (1): Firstly, lesions of MPOAdl/BSTv disrupt maternal behavior in both postpartum mothers and pup-sensitized virgin female rats. Secondly, during performance of maternal behavior, MPOAdl/BSTv neurons are activated and express transcription factors such as c-Fos and FosB. Lastly, MPOAdl/BSTv neurons express hormone receptors that are involved in female reproduction such as oxytocin, prolactin and estrogen. Application of such hormones into MPOA may enhance maternal behavior. The precise anatomical definition and delineation of MPOAdl/BSTv, however, remain to be determined. Our recent report has suggested that the densest distribution of c-Fos positive neurons in response to pup exposure in maternal virgin mice coincides with the anterior commissural nucleus (ACN) and its rostral extension (rACN) (2). ACN is the third largest population of oxytocin neurons, and is located just on the border of MPOAdl and BSTv in both rats and in mice (3, 4). ACN also contains many other types of neurons, and these non-oxytocinergic neurons may be more directly involved in the execution of maternal behavior than the oxytocinergic neurons. To identify the roles of these neuron subpopulations, we are currently

investigating the effects of excitotoxic amino acid lesions of ACN or in the adjacent areas on various aspects of maternal behavior, including retrieval, nest-building, nursing and maternal aggression. We will also discuss the possible regulatory role of oxytocin neurons in coupling female reproductive events and maternal behavior.

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P36

Physiological role of acid-sensing ion channels in the identified rat hypothalamic vasopressin neurons of the supraoptic nucleus

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Body fluid balance requires the release of arginine vasopressin (AVP) from the neurohypophysis. The hypothalamic supraoptic nucleus (SON) is one of the major sites for the synthesis of AVP, and somatodendritically or at the nerve terminals, AVP secretion is controlled by the electrical activities of magnocellular neurosecretory cells (MNCs). Acid-sensing ion channels (ASICs) are neuronal voltage-insensitive cationic channels activated by extracellular acidification. ASICs are known to be modulated by lactate (La^-) which reduce extracellular divalent ion. We hypothesize that ASICs might modify neuronal function through a La^- which is generated during local hypoxia and also during anaerobic glycolysis resulting from osmotic stimulation in the SON. Here we show that the functional ASICs are expressed and enhanced by La- in the rat isolated SON MNCs expressing AVP-enhanced green fluorescent protein transgene (1, 2). Moreover, the increase of La^- production was specifically observed in the SON under the hyperosmotic conditions. These results suggest that the possibility that ASICs- La^- interaction in the SON has an important role in the regulatory mechanism of body fluid homeostasis.

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P37

Behavioral and anatomical characterization of mouse pup response to maternal transport

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An infant's behavior is adapted to the type of parental care it receives from its caregiver. Therefore, the parent-infant relationship is a reciprocal process. For example, when a human mother carries her baby, the baby tends to calm down and cling to the mother. In many mammals with altricial young, a mother carries her young by oral-grasping. When a rat mother picks up a pup by its dorsal skin to transport it, the pup becomes immobile and adopts a compact posture which is characterized by flexion of hindlimbs (1). These characteristic "pup responses to transport (PRT)" may be considered a form of attachment behavior, since it facilitates maternal transport. We studied PRT in mice by behavioral and anatomical analyses. First, we investigated the ontogeny of PRT during the first 20 days after birth. The experimenter can easily induce the PRT by manually grasping the pup's back and lifting it up. From postnatal day (P) 3, almost all pups became immobile when they were picked up. In addition, pups begin to maintain the hindlimb flexion from P8 onward. After P17, most of the pups do not become immobile nor maintain the hindlimb flexion. Therefore, immobilization response of PRT has a clear time window between P3 and P16. In addition to immobilization, hindlimb flexion response is added P8 onward to further help the maternal transport of the growing pup. Next, we explored the brain regions activated during expression of PRT by c-Fos immunohistochemistry. Our preliminary results showed that c-Fos immunopositive neurons were significantly increased in some brain stem nuclei of pups in which PRT was repeatedly induced. Our working hypothesis for PRT-regulation and the interaction of its neural system with the brain's arousal system (norepinephrine and neuropeptides) will be discussed.

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P38

Glial control of synapse-specific actions of retrograde endocannabinoids in rat hypothalamic supraoptic nucleus

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We have reported a synapse-specific mechanism of glucocorticoid regulation of hypothalamic magnocellular neurons. Thus, glucocorticoid-induced retrograde endocannabinoid (eCB) actions are spatially restricted to glutamate synapses, and do not spill over onto neighboring GABA synapses, despite the expression of functional CB1 receptors at GABA synapses. The functional restriction of eCBs to glutamate synapses may be the result of extracellular buffering of the messengers by astrocytes. We tested this by recording whole-cell synaptic currents

in hypothalamic slices following manipulations to reduce glial buffering mechanisms, including dehydration-induced glial retraction and blocking glial metabolism with fluorocitrate. Under these conditions of attenuated glial function, glucocorticoids elicited an eCB suppression of both glutamate and GABA release, suggesting spillover of eCBs onto GABA synapses. In the dehydration model, the glucocorticoid-induced suppression of GABA release, but not glutamate release, was prevented when extracellular viscosity was increased and diffusion retarded with the large neutral molecule dextran. These results suggest that restriction of the actions of glucocorticoid-induced endocannabinoids to glutamate synapses is controlled by astrocytes, and that attenuation of glial buffering leads to spillover of the endocannabinoids onto GABA synapses. Astrocytes, therefore, limit the actions of both orthograde and retrograde messengers spatially, and glial plasticity promotes synaptic crosstalk of messengers emanating from both presynaptic and postsynaptic cells.

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P39

Synchronized non-stochastic quantal release of GABA: a novel mechanism for burst generation and synchronization

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Quantal synaptic release is generally stochastic and therefore is not thought to mediate information relay in neuronal circuits. Using whole-cell recordings in acute hypothalamic slices, we recorded endogenous bursts of inhibitory postsynaptic currents (IPSCs) in magnocellular neurons caused by non-stochastic GABA release in acute hypothalamic slices. Bursts of IPSCs were unaltered by blocking spiking activity with tetrodotoxin, indicating that they were spike-independent and comprised, therefore, of miniature IPSCs (mIPSCs); the IPSC burst incidence, however, decreased by ~80% with spike blockade, suggesting that the probability of burst generation was attenuated in the absence of presynaptic spiking activity. Spontaneously occurring endogenous bursts of inhibitory postsynaptic potentials and bursts simulated experimentally by intracellular current injection were capable of both triggering and terminating spike trains, suggesting that they are capable of shaping spike bursts in magnocellular neurons. Unexpectedly, some IPSC bursts recorded in pairs of magnocellular neurons were highly synchronized in onset, while intra-burst IPSCs were not synchronized. The synchronized IPSC bursts were more uniform in their duration and intra-burst frequency than the unsynchronized bursts recorded in the same paired cells, which was consistent with the synchronized IPSC bursts being generated at “shared” GABA synapses between the paired neurons. This synchronized, non-stochastic quantal inhibitory synaptic input represents a novel form of synaptic transmission that dissociates postsynaptic activation from presynaptic spiking activity. It also provides a potentially robust mechanism of spike burst generation, termination and synchronization that may play an important role in the coordination of electrical activity among magnocellular neurons and the pulsatile release of oxytocin and vasopressin.
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P40

Minocycline prevents osmotic demyelination by inhibiting the activation of microglia in rats.

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Osmotic demyelination syndrome (ODS) is a serious demyelination disease commonly associated with a rapid correction of chronic hyponatremia. Although its pathogenesis remains to be clarified, we previously reported that dexamethasone (DEX, 2mg/kg bw) could prevent the disruption of the blood-brain barrier (BBB) that is caused by the rapid correction of hyponatremia and its associative demyelination in rats. We also reported that microglia accumulated in the demyelinative lesions might play a detrimental role in the pathogenesis of ODS by producing proinflammatory cytokines. On the other hand, minocycline (MINO) is known to downregulate the activity of microglia. In the present study, we investigated whether MINO could protect against osmotic demyelination in rats and compared the therapeutic efficacy of MINO and DEX. Hyponatremia was induced in rats by liquid diet feeding and dDAVP infusion. Seven days later, the hyponatremia was rapidly corrected by injecting a bolus of hypertonic saline intraperitoneally. Rats subjected to this treatment displayed serious neurological impairment and approximately 80% died within 5 days of the correction. On the other hand, rats that were treated with MINO (45 mg/kg bw, 0, 12 and 24 h after hypertonic saline injection) exhibited minimal neurological impairment and all were alive within 5 days. A MINO injection at 24 h after the rapid correction when BBB breakdown occurred abrogated the exacerbation of neurological symptoms, while DEX injections after the BBB breakdown had no effect. The dose-dependent study showed the preventive effect of MINO on neurological symptoms was pronounced than that of DEX. Histological analysis showed that MINO attenuated the early demyelination and inhibited the subsequent development of demyelination. MINO also decreased the accumulation of microglia in the demyelinative lesions. In addition, extravasations of IgG and Evans blue dye in the brain were observed in the MINO treated group, indicating that MINO did not prevent the disruption of BBB. Real-time PCR and immunohistochemical analysis showed that MINO inhibited the activity of microglia and the expression of the inflammatory cytokine (i.e., IL-1b, iNOS, and TNFa) and the MCP-1 in microglia after the rapid correction of hyponatremia. In conclusion, our data showed MINO inhibited the activation and accumulation of microglia in the demyelinative lesions, thereby preventing the development of osmotic demyelination and suggest MINO is more efficient than DEX in prevention of ODS.

P41

The role of the vasopressin V1b receptor in the HPA axis response to stress: molecular and pharmacological studies

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The role of arginine vasopressin (Avp) as an ACTH secretagogue in the hypothalamic-pituitary-adrenal (HPA) axis is mediated by the Avp V1b receptor (V1bR) located on anterior pituitary corticotropes. Using our mouse V1bR knockout (KO) model we have previously shown that an intact V1bR is required for a normal HPA response to a variety of acute stressors[1-3]. To further investigate the role of the V1bR in the HPA response to stress, we have compared the plasma ACTH and corticosterone (CORT) responses to acute restraint, forced swim and shaker stress in adult V1bR KO and wild-type mice that have been pre-treated with a novel V1bR antagonist (Schering-Plough). The diminished plasma ACTH levels in acutely stressed V1bR KO mutants were indistinguishable from those observed in similarly stressed, V1bR antagonist-treated, wild-type mice. In contrast, the V1bR is not required for a normal CORT response to acute restraint, forced swim or shaker stress. There was no adaptation of the ACTH response to repeated restraint or forced swim in wild-type mice - on the other hand, the adaptation observed to repeated shaker stress was absent in V1bR KO animals.
Our studies demonstrate the importance of the V1bR in the HPA response to most acute stressors and at least one chronic stressor. Our studies also suggest that loss or blockade of the V1bR is not compensated for by other ACTH secretagogues. Furthermore, we have shown a clear dissociation between plasma ACTH and CORT levels following acute restraint, forced swim and shaker stress. It is clear that the nature and/or severity of the stress is a critical consideration when interpreting the HPA response to stress as factors that regulate the CORT response to stress may be dynamically influenced by different types of stressors.
Studies on the physiology of the V1bR *in vivo* have been hampered by the lack of specific ligands. We have now developed and refined an autoradiographic protocol using a radiolabelled-V1bR antagonist - for the first time we can now specifically image the V1bR to determine where it is expressed and how it is regulated. The V1bR is predominantly expressed in the pituitary and CA2 region of the hippocampus of rats and mice (Fig.1) - radiolabelled-V1bR antagonist binding is absent in V1bR KO mice. The hippocampus may be the anatomical substrate for V1bR effects on aggression, anxiety, social behaviour and/or episodic memory.

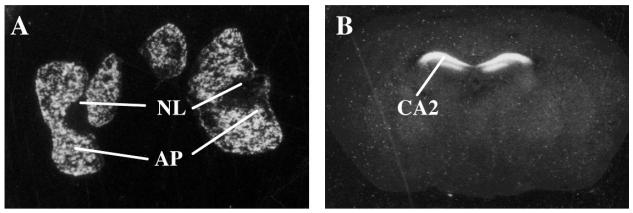

Fig.1. Autoradiography of adult, male rat (Sprague-Dawley) pituitary (A) and V1bR KO wild-type mouse brain (B) using a radiolabelled-V1bR antagonist. Note intense labeling of anterior (AP) but not neural lobes (NL) of pituitary gland and CA2 region of the hippocampus (CA2). The photomicrographs are pseudocolour images taken directly from the autoradiographic film.

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SUPERVISORS; DAVID MURPHY AND SONG YAO THE FRACTALKINE RECEPTOR CX3CR1 IS UP-REGULATED IN THE BRAIN OF HEART FAILURE RATS.

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The onset and progression of chronic heart failure (CHF) is accompanied by a multitude of neural-humoral changes. For example, striking increases in sympathetic activity and increased circulating levels of factors, including inflammatory cytokines, are hallmarks of CHF (Deswal et al 2001, Ericson et al 1997). Indeed, it has been suggested that circulating levels of inflammatory cytokines correlate well with disease severity and is a robust predictor of poor prognosis (Deswal et al 2001). While circulating cytokines, via their actions on the microvasculature, have been investigated in the past (Ericson et al 1997), relatively little is known about the cytokines produced within the brain, particularly in relation to disease states such as CHF. Here, we used quantitative RT-PCR gene profiler arrays to screen for changes in the expression of 84 cytokine related genes within the brainstem of sham operated (control) and CHF rats. We identified significant ($p<0.05$, one-way ANOVA) changes of 2-fold or greater in 41 out of 84 genes screened. One gene of particular interest is fractalkine (3.48-fold increase in CHF compared to sham operated rats, $p=0.0004$), also known as CX3CL1, as this cytokine has previously been shown to play a significant role in modulating neuronal function (Francis, Zhang et al. 2004). Immunohistochemistry revealed a robust and significant increase in the number of neurones expressing the receptor for CX3CL1, CX3CR1, in the brain stem and hypothalamus of CHF rats, particularly in the commissural nucleus tractus solitarius (329±29 vs. 400±7, $n=5$, $p<0.05$) and in the spinally projecting regions of the paraventricular nucleus (226±21 vs. 303±21, $p<0.05$) as well as the magnocellular region (132±4 vs. 170±6, $p<0.05$). Furthermore, doubleimmunoflourescence histochemistry revealed that while CX3CL1 is expressed both in neurones and glia, the receptor, CX3CR1, appears to be present only on neurones. Our findings suggest that cytokines, and in particular CX3CL1, may play a role in modulating neuronal function and, hence, might be responsible for mediating the neural-humoral changes observed in CHF.

Personal statement

I am Currently in my second year of my PhD and my project is based on looking at the effects of proinflammatory cytokines (PICs) in the central nervous system (CNS) in heart failure. PICs have been shown to be expressed in the CNS; I focus on a particular cytokine called fractalkine and its receptor, fractalkine receptor. I have found that fractalkine receptor is significantly up regulated in the dorsal paraventricular nucleus at 4 weeks chronic heart failure vs. sham rats and by 8 weeks, fractalkine receptor is significantly up regulated in the parvocellular and magnocellular paraventricular neucles in chronic heart failure rat vs. sham rats. Fractalkine receptor is additionally up regulated in the commissural nucleus tractus solitarius in the 8-week chronic heart failure group vs. the 8 week sham group. These regions are interesting in heart failure as they are cardiovascular centres, and I have injected fractalkine into the nucleus tractus solitarius which causes a significantdrop in mean arterial pressure by an average of 15mmHg in sham rats. This is a very exciting part of my project and I will like to inject fractalkine in the paraventricular nucleus to observe a response. I would also like to carry out these injections on heart failure rats, which will enable a comparison between sham and chronic heart failure rats. PICs may exert their effects by acting in synergy with other systems such as the rennin-angiotensin system and reactive oxygen species. I would like to find out the interactions of these systems in the progression of heart failure. Having the opportunity to attend the WCNH conference, I believe will help me interact with fellow scientists and hopefully lead to collaborations. I also believe having this chance to attend will help improve my skills in communicating academically and learning in more depth about research currently going on in and around the field.

P43

Spontaneous cell fusion in cultured isolated fetal rat supraoptic neurons

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Some neuroendocrine cells are known to form syncytia (1), which may contribute to synchronous neuronal activity resulting in the bolus release of the hormone. Here we investigated whether or not hypothalamic supraoptic neuroendocrine neurons spontaneously form syncytia during culture. Supraoptic region was punched out from the base of the brain of rat fetus (embryonic day 20). Cells were mechanically and enzymatically isolated from the specimen, plated at a density of $1\times10^6/cm^2$, and cultured with Neurobasal medium containing B-27 supplement. Cortical, hippocampal, and spinal neurons were also cultured at the same time. In some experiments, cells were stained with anti-microtubule-associated protein 2 (MAP2, neuronal cell marker) antibody, anti-vasopressin antibody, or propidium iodide (nuclei marker). Cellular morphology and fluorescence were observed under video-microscope or confocal microscope. Within 24 h of culture, syncytia with multiple nuclei (2?20 nuclei) were observed in supraoptic neurons but not cortical, hippocampal, or spinal neurons. In some syncytia, nuclear fusion was also observed. Organelles were moving throughout the cytoplasm of syncytia. Fluorescence staining revealed that all syncytia were neuronal cells and some syncytia were vasopressin-immunoreactive. Syncytia with 2?5 nuclei extended their neurites. These observations indicate that cell fusion in supraoptic neurons may be involved in development of supraoptic neuroendocrine cell morphology and function.

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P44

Nociceptive stimulation causes exaggerated response of arginine vasopressin-enhanced green fluorescent protein gene expression in rats

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Abstract
Nociceptive stimulation causes neuroendocrine responses such as arginine vasopressin (AVP) release and activation of the hypothalamo-pituitary adrenal (HPA) axis. We have generated novel transgenic rats expressing an AVP-enhanced green fluorescent protein (eGFP) fusion gene, and we examined the effects of nociceptive stimulation on the expression of the AVP-eGFP gene in the hypothalamus after subcutaneous injection of formalin or saline into the bilateral hind paws in transgenic rat. We measured sodium concentrations and AVP levels in plasma and the changes of AVP mRNA, eGFP mRNA, and AVP heteronuclear (hn) RNA in the supraoptic nucleus (SON) and the paraventricular nuclei (PVN) over the time course of injection. Then, we observed eGFP fluorescence in the SON, the PVN, median eminence (ME) and posterior pituitary gland (PP) after the injection. The plasma concentrations of AVP were significantly increased 15 min after the formalin injection compared with

saline-injected or untreated rats without affecting the plasma sodium concentrations, and returned to the same levels of saline-injected or untreated rats at 2 and 6 h after the injection. Although AVP mRNA levels in the SON and the PVN did not change, eGFP mRNA levels in the SON and the PVN were significantly increased at 2 and 6 h. The AVP hnRNA in the SON and the PVN were also significantly increased at 2 h and both of 15 min and 2 h, respectively. The eGFP fluorescence in the SON, parvocellular and magnocellular divisions of the PVN, internal and external layers of ME, and PP was markedly increased in formalin-injected rats compared with saline-injected or untreated rats, and especially increased in parvocellular parts of the PVN. These results suggest that AVP-eGFP in the hypothalamus can change rapidly after acute nociceptive stimulation, thus, AVP-eGFP transgenic rat is a unique animal to study acute stress responses in AVP neuron of the hypothalamus.

P45

Intracellular localization of synaptophysin in cultured rat hypothalamic supraoptic neurons

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Synaptophysin is a membrane-bound protein of synaptic and neuroendocrine secretory vesicles. In the present study, intracellular localization of synaptophysin was studied in cultured rat hypothalamic supraoptic neurons using immunocytochemical and green fluorescent protein (GFP) labeling techniques. Supraoptic neurons were isolated from rat embryos (E20) and cultured for 3 days. For immunocytochemistry, neurons were fixed and fluorescently stained with antibodies against synaptophysin, vacuolar H^+_ATPase, vasopressin, and lysosome-associated membrane protein 2 (LAMP-2). For GFP labeling, neurons were transduced with baculoviral vector encoding GFP-synaptophysin. Cellular fluorescence was observed with a confocal microscope. Immunocytochemistry revealed that synaptophysin was located in vesicles present in cell bodies, neurites, and neurite endings. Neurite endings exhibited the highest density of synaptophysin-immunoreactive vesicles. Synaptophysin was co-localized with vacuolar H^+_ATPase (a proton pump present on membranes of lysosomes, synaptic vesicles, and secretory granules) and vasopressin, but not with LAMP-2. GFP-tagged synaptophysin in living cells was distributed similar to synaptophysin immunoreactivity. No co-localization of GFP-tagged synaptophysin and lysosomes stained with Lysotracker was observed. These observations indicate that synaptophysin in supraoptic neurons is localized in neuroendocrine secretory vesicles but not lysosomes in supraoptic neurons.

P46

Vasopressin has antiapoptotic actions in primary cultures of hippocampal neurons

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The recent demonstration that VP prevents serum deprivation-induced cell death in the neuronal cell line, H32, suggests that the peptide have neuroprotective actions. This hypothesis was tested in primary cultures of hippocampal neurons by examining the ability of VP to prevent apoptosis and signaling pathways involved in this effect. Deprivation of trophic factors for 24 hours (by replacing the B-27 supplement with 0.1% BSA in the culture medium) or glutamate exposure decreased neuronal cell viability and an increased caspase-3 activity, consistent with apoptotic cell death. This effect was significantly reduced by addition of 10nM VP, suggesting that VP exerts anti-apoptotic effects in neurons. This was confirmed by the ability of VP to prevent trophic factor deprivation or glutamate-induced Tdt-mediated dUTP nick-end labeling (TUNEL) staining neurons. The protective effect of VP was completely blocked by V1 receptor antagonist, (Phenylac^{1},D-Tyr(Et)^{2},Lys^{6},Arg^{8},des-Gly^{9})-Vasopressin, indicating that it is mediated via V1 VP receptors. The anti-apoptotic effect of VP in neurons was mediated by mitogen activated protein (MAP) kinase and extracellular signal-regulated kinases (ERK) signaling pathway, since co-incubation with VP and the selective MAPK inhibitor, U0126, prevented the inhibitory action of VP on nutrient deprivation-induced caspase-3 activity and TUNEL staining neurons. These data show that VP has anti-apoptotic actions in neurons, an effect which is mediated by the MAPK signaling pathway. The study supports the view that VP plays a role as a neuroprotective agent in the brain.

This work was supported by the NICHD, NIH, Intramural Research Program.

P47

Oxytocin-induced analgesia is not mediated by the oxytocin receptor, but rather by the vasopressin-1A receptor: evidence from oxytocin- and vasopressin-receptor knock-out mice

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Oxytocin (OXT) and arginine vasopressin (AVP) are two closely related nineamino acid neuropeptides synthesized in the paraventricular and supra-optic nuclei of the hypothalamus. They are either transported to the posterior pituitary and secreted into the blood stream to exert a variety of hormonal effects, or released into several regions of the central nervous system (CNS), where they act as modulators of neuronal transmission. Recently, the actions of OXT and AVP in the CNS have received increasing attention and major discoveries have been made with respect to the role of these peptides for the regulation of complex social and sexual behavior in mammals. There is also a growing body of literature describing the analgesic effects of OXT and AVP in both human and rodent species. Indeed, OXT has been reported to be analgesic in a variety of pain tests when administered directly into the brain, the spinal cord or systemically. These pharmacological data fit well with the enrichment of OXT receptor binding sites in the substantia gelatinosa of the spinal cord.
In the CNS, OXT and AVP exert their effects through binding to the oxytocin receptor (OTR) or the 1A subtype of the vasopressin-receptor (V1AR), respectively. It is well documented that the strong sequence homology of OXT and AVP can lead to the activation of the V1AR by OXT, and vice-versa. Here, we characterized the pain phenotype of mutant mice possessing nonfunctional OTR or V1ARs. Surprisingly, we found that OTR knock-out mice display a pain phenotype identical to their wildtype littermates. Moreover, systemic administration of OXT dose-dependently produced analgesia in both OTR knock-out and wildtype

mice in two different pain tests: the radiant heat paw-withdrawal test for baseline thermal nociception and the formalin assay for inflammatory pain. In contrast, OXT-induced analgesia was completely absent V1AR knock-out mice. These observations suggest that the analgesic effects of OXT are mediated by V1AR rather than by OTR. We are in the process of mapping the distribution of OXT AVP binding site in mouse spinal cord.
Centrally, both, OXT and AVP elicit a strong scratching response. Here, we report that OXT-induced scratching is present in both OTR wild-type and knock-out mice, but absent in V1AR knock-out animals. These results suggest that in mice in addition to analgesia, other central actions of OXT are also mediated through the V1AR.

P48

Involvement of orexin-A on micturition reflex in normal bladder but not cyclophosphamide-induced cystitis in rats

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Purpose: The purpose of the present study was to investigate the effect of orexin-A in the spinal cord on the bladder function in normal rats and cyclophosphamide (CYP)-induced cystitis rat models.
Methods: The effects of intrathecal (i.t.) injection of orexin-A (0.01, 0.1 and 1.0 nmol) on bladder function were examined during continuous infusion cystometrogram (CMG) in urethane anesthetized normal and CYP-induced cystitis rats. The effects of i.t. injection of selective orexin-1 receptor (OXR1) antagonist SB334867 (10 nmol) on orexin-A-induced bladder overactivity in normal rats and SB334867 (10 and 30 nmol) on changes in bladder function in normal and CYP-induced cystitis rats were investigated. The effects of intravenous (i.v.) injection of orexin-A (0.3 and 1.0 nmol) on micturition reflex were also investigated in normal rats.
Results and Conclusion: I.t. injection of orexin-A (0.1 and 1.0 nmol) significantly decreased the intercontraction intervals (ICI) in normal and CYP-induced cystitis rats. I.t. injection of SB334867 (10 nmol) significantly increased the ICI of orexin-A induced overactive bladder in normal rats and i.t. injection of SB334867 (30 nmol) also increased the ICI in normal rat bladder. However, in CYP-injected cystitis rat models, i.t. injection of SB334867 did not change the bladder function. I.v. injection of orexin-A failed to affect the bladder function in normal rats. These results indicate that orexin-A in the spinal cord activates the micturition reflex via OXR1 in normal rats. In addition, OXR1 antagonist did not have any effect on micturition reflex in CYP-induced cystitis rat models.

P49

Chronic social defeat stress induces hyperthermia in rats

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Acute psychological stress such as cage switch stress is known to induce a transient increase in core body temperature (Tc) in laboratory animals. However, studies on the effect of repeated stress on Tc are still limited. Therefore, we observed changes in Tc of the male Wistar rats after animals were subjected to one-hour social defeat daily for four weeks. The Tc was monitored continuously by using a telemetry system. On the first day, social defeat stress induced a transient increase in Tc up to 1.5deg C, which returned to the baseline Tc within three hours. After four-week stress session, the stressed rats showed higher Tc than non-stressed rats in both the light and the dark periods. This hyperthermia was observed for one week after cessation of social defeat. The present study suggests that repeated social defeat stress induces chronic hyperthermia in rats.

P50

Expression of Rho-guanine nucleotide exchange factor ECT2 in the adult and developing pituitary

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Previously, we performed *in vivo* RNAi screening to identify Drosophila genes required for the development of the embryonic nervous system (1). From a library of double stranded RNAs we identified 43 genes whose role in embryonic nervous system development has not been described. We identified their mouse orthologue and then performed RT-PCR analysis to identify the genes that were expressed in the adult and embryonic mice brain. Based on such expression profiling experiments, we found that Ect2, a GEF for Rho GTPases, was expressed in the adult pituitary, but not in other brain region tested. Ect2 is well known for its involvement in cytokinesis, but the exact role of Ect2 in the nerveous and endocrine systems is not known.
To assess the above question, we examined Ect2 expression and localization in the postnatal pituitary. From the western blotting analysis, we found Ect2 expression in the postnatal mouse pituitary decreases with age development. Expression of Ect2 coincided with postnatal pituitary growth. Immunostaining of Ect2 showed expression in the intermediate and anterior pituitary. Cells expressing Ect2 incorporated EdU, proliferation marker. Taken together, Ect2 plays a role in proliferation of cells in anterior and intermediate pituitary. Our results suggest that Ect2 functions in cytokinesis of cells in the anterior and intermediate pituitary.

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P51

Expression and characterization of human oxytocin receptor in HEK293 cells

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Oxytocin (OT) regulates social and affective behaviors through activation of its cell-surface receptor. Although OT supplementation has become a major concern in the treatment of neuropsychiatric diseases such as autism spectrum disorders, fundamental mechanisms underlying the OT action have not fully been elucidated. We therefore developed a heterologous expression system for studying the molecular and cellular mechanisms mediated by the human oxytocin receptor (hOTR). Complementary DNA (cDNA) encoding hOTR was amplified from commercially available human brain RNA by reverse transcription- polymerase chain reaction, and was verified by nucleotide sequencing. The obtained cDNA was then cloned into pcDNA3.1, a mammalian expression plasmid, and was transiently transfected into the human embryonic kidney cell line HEK293. Immunocytochemical staining detected hOTR-immunoreactivity (IR) in HEK293 cells transfected with the hOTR-expression plasmid; in contrast, both pcDNA3.1-transfected and untransfected cells did not exhibit hOTR-IR at all. We next examined agonist-induced internalization of hOTR using antibody against the *N*-terminus of hOXTR. After stimulation with OT at the final concentration of 100 nM, hOTR-IR at the cell surface continued to decrease over a period of 60 min; in parallel, perinuclear IR became intense, and occasionally formed dense aggregates. Furthermore, we constructed an expression plasmid for hOTR fused to enhanced green fluorescent protein (EGFP) at its C-terminus, and transfected into HEK293 cells as well. In EGFP fluorescence-positive cells, OT elicited a transient increase in intracellular calcium concentration, and as observed in the hOXR-expressing cells, induced perinuclear aggregates. These observations indicate that these hOTR- and hOTR-EGFP-expressing cells could be used for analysis of desensitization and internalization of the hOTR. Also, these cells would serve as a useful tool for evaluation of psychotomimetics targeting the hOTR.

P52

A case of simultaneous presentation of central diabetes insipidus and the syndrome of inappropriate antidiuretic hormone secretion

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A 65-year-old Japanese male was admitted to our hospital for an operation of sphenoid sinus mucocele. Preoperative endocrinological examinations revealed partial anterior pituitary dysfunction and central diabetes insipidus (DI). After the endoscopic transsphenoidal marsupialization, though anterior pituitary dysfunction improved, DI remained. 1-desamino-8-D-arginine vasopressin (dDAVP) was administered and his daily urine volume was normalized, however, serum sodium concentration promptly fell down to 115 mmol/l. Also we found that the plasma arginine vasopressin (AVP) levels were not fully suppressed under the hyponatremia. Though we gradually reduced dDAVP, serum sodium levels did not recovered to normal range. When

we finally discontinued dDAVP, severe polyuria and hypernatremia reappeared. After that, his serum sodium concentrations continued to swing between 120 and 154 mmol/l depending on the doses of dDAVP, though the plasma AVP levels were almost always fixed around 1.0 pg/ml. According to the findings that insufficient AVP release was observed at hyperosmolar state, relatively high levels of plasma AVP continued under the hypoosmolar state, and any signs of hypovolemia were not observed, we reached the final diagnosis of simultaneous presentation of DI and the syndrome of inappropriate antidiuretic hormone secretion.
To investigate whether the baroregulation of AVP release was intact, we examined the effect of hypotensive stimuli on plasma AVP. Though intravenous infusion of nicardipine decreased mean arterial pressure by more than 30%, we could not find any increase in plasma AVP levels.
These findings suggest that the predominant lesion of our case is not located around osmoreceptor itself but AVP producing nuclei which were functionally isolated from the neurological input from central osmoreceptor and peripheral baroreceptors.

P53

The effects of cold stress and beta blocker (propranolol) on LH and estrogen in presence and absence of locus coeruleus in female rat

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In this study we have examined the effect of cold stress on LH and stradiol concentration in presence and absence of locus coeruleus and beta blocker (propranolol) in the female rat.
110 female Wistar rat weighted 160-200g were divided into 11 groups.
A) Intact animals:
1. intact group **2.** cold stress group **3.** cold stress + saline **4.** cold stress + 4mg/kg propranolol **5.** cold stress + 7mg/kg propranolol **6.** cold stress +10mg/kg propranolol.
B) Surgical animals:
1. sham surgical group **2.** lesion surgical group **3.** lesion+cold stress **4.** lesion+cold stress+saline **5.** lesion+cold stress + 7 mg/kg propranolol.
Locus coeruleus were lesioned using 10 mA electrical current for 1s. Cold stress exposure were applied during proestrous period for all animals. Animals were exposured to at 4?C for 20 minute.
Results & Discussion
Experimental results showed that
1) Acute cold stress has no effect on LH in intact or locus coeruleus lesioned group.
2) Acute cold stress has no effect on estradiol in intact or locus coeruleus lesioned group.
3) propranolol at 7mg/kg were increased the levels of LH in intact animals (p?0.01), but it has no effect on LH in locus coeruleus lesioned group.
4) Propranolol has no effect on levels of estradiol in intact or locus coeruleus lesioned group.
5) Locus coeruleus lesion increases LH in the female rats but cold stress can not alter it in comparisons with sham animals (p?0.01).
6) Locus coeruleus lesion caused a decrease in estradiol level in the female rat (p?0.001).
It seems that, this amount of cold stress has no effect on LH and estradiol at late proestrous phase in the intact or locus coeruleus lesioned group. Propranolol at different doses has a distinct effect on the noradrenergic system and increases LH but not estradiol. So locus coer-

uleus plays an important role in secretion of sexual hormones.
Key Words: Acute cold stress, LH, Estradiol, Locus coeruleus, Propranolol

P54

Administration of desmopressin suppressed aggregate formation in vasopressin cells of a mouse model for familial neurohypophysial diabetes insipidus

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Familial neurohypophysial diabetes insipidus (FNDI), an autosomal dominant disorder, is mostly caused by mutations in the gene encoding neurophysin II (NPII), the carrier protein of arginine vasopressin (AVP). We generated a mouse model for FNDI by knocking-in a point mutation (Cys98stop) in the NPII gene (1). The mice manifested progressive polyuria, and histological analyses revealed that aggregates were formed in the endoplasmic reticulum (ER) of AVP cells in the supraoptic nucleus (SON). While it is possible that AVP precursors were accumulated in the ER as the aggregates, they were not immunostained with antibodies for mutant NPII, normal NPII or AVP. In the current study, we examined whether suppression of AVP synthesis could prevent the aggregate formation. Two month-old female heterozygous mice were administered either desmopressin (dDAVP) or vehicle (control) subcutaneously with osmotic minipumps for 1 month. Urine volume and water intake were significantly reduced and urine osmolality was increased in the dDAVP group compared to the control during the treatment. One month after starting the treatment, the levels of blood hematocrit and AVP mRNA expression in the SON were significantly lower in the dDAVP group compared to the control. Furthermore, the number and size of the aggregates in the SON were significantly decreased in the dDAVP group compared to the control. In separate experiments, we monitored changes in the phenotype after one-month administration of dDAVP or vehicle. Urine volume remained significantly less for 10 days after cessation of dDAVP treatment compared to the control. These data demonstrated that dDAVP administration suppressed the aggregate formation in the AVP cells of SON by decreasing AVP synthesis, and suggested that the progression of the phenotype was closely related with the aggregate formation in the ER of the AVP neurons in FNDI.

(1) Hayashi M, et al., Progressive polyuria without vasopressin neuron loss in a mouse model for familial neurohypophysial diabetes insipidus. Am J Physiol Regul Integr Comp Physiol 296: R1641-R1649, 2009.

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Effects of high salt diet on the progression of polyuria in a mouse model for familial neurohypophysial diabetes insipidus

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Familial neurohypophysial diabetes insipidus (FNDI) is a rare disease which is inherited in

an autosomal dominant manner. While polyuria due to decreases in vasopressin release appears several months or years after birth, there is heterogeneity in the age of onset of the polyuria among patients even with the same mutation, suggesting other than genetic factors might influence the progression of the disease. This study was conducted to elucidate whether the amount of sodium intake affects vasopressin release as well as the progression of polyuria in knock-in mice expressing mutant NPII (Cys98stop) that causes FNDI in humans. The mice manifested progressive polyuria, and aggregates were formed in the endoplasmic reticulum of vasopressin cells in the supraoptic nucleus (1). The male heterozygous as well as wild-type mice were fed either 0.2% Na or 2.0% Na diet for 6 months starting at 1-month-old. Urinary AVP excretion in the 2.0% Na group was significantly increased compared to that in the 0.2% Na group in wild-type mice throughout the experiment. On the other hand, urine AVP excretion was significantly increased in the 2.0% Na group compared to the 0.2% Na group at 2- and 3- month-old, but it was gradually decreased thereafter in the heterozygous mice. While there was no time-course effect on urine volume in the 2.0% Na and 0.2% Na groups in wild-type mice, urine volume increased progressively in the 2.0% Na group compared to the 0.2% Na group in the heterozygous mice. Immunohistochemical analyses revealed that the number of inclusion bodies in the AVP cells in the supraoptic nucleus was significantly increased in the 2.0% Na group compared to the 0.2% Na group in the heterozygous mice. These data demonstrated that excess in salt intake stimulated AVP neurons and accelerated the progression of the polyuria in a mouse model for FNDI.

(1)Hayashi et al., Progressive polyuria without vasopressin neuron loss in a mouse model for familial neurohypophysial diabetes insipidus. Am J Physiol Regul Integr Comp Physiol 296: R1641,2009

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Proinflammatory and Neurotrophic Responses of Microglia and Astrocytes in a Rat Model for Osmotic Demyelination Syndrome

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Osmotic demyelination syndrome (ODS) is a serious human demyelinating disease in the central nervous system usually caused by rapid correction of chronic hyponatremia due to syndrome of inappropriate antidiuretic hormone secretion. In ODS, several studies reported microglial accumulation in demyelinative lesions, and we previously revealed these microglia expressed proinflammatory cytokines. It has been reported that proinflammatory cytokines and neurotrophic factors secreted by microglia and astrocytes are involved in the pathogenesis of demyelinative or neurodegenerative diseases, such as multiple sclerosis or Parkinson's disease. Therefore, to clarify the roles of glias in the pathogenesis of ODS, we examined the time-dependent changes in distributions, morphology, proliferation, mRNA and protein expression of proinflammatory cytokines and neurotrophic factors in microglia and astrocytes at 2 days (early phase) and 5 days (late phase) after the rapid correction of hyponatremia in rats. The number of microglia time-dependently increased in demyelinative lesion and microglia expressed tumor necrosis factor-?, IL-1?, IL-6 and inducible nitric oxide synthase with a proliferation at early phase. Microglia also expressed leukemia inhibitory factor, which were

known as a neurotrophic factor, with a time-dependent increase and phagocytosed myelin debris at late phase. The number of astrocytes time-dependently increased around demyelinative lesion and astrocytes expressed nerve growth factor and glial cell line-derived neurotrophic factor with a proliferation at late phase. Moreover, increased astrocytes came closer and extended processes to demyelinative lesion at late phase. These results suggest that the role of microglia in the pathogenesis of ODS time-dependently alters from detrimental to protective and that astrocytes have a protective role at late phase, and further suggest that modulation of excessive proinflammatory responses in microglia at early phase may become a therapeutic target for ODS.

P57

Yokukansan reduces stress-induced hyperthermia in mice

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Yokukansan (YKS, *Yi-gan san* in Chinese) is a traditional herbal medicine, which was developed in 1955 by Xue Kai as a remedy for restlessness and agitation in children. YKS is demonstrated to be effective for treating behavioral and psychological symptoms of dementia in the elderly and behavioral and psychological symptoms, including low mood, impulsivity, and aggression, of borderline personality disorder. Animal studies suggest that YKS may have anxiolytic, anti-depressant and anti-nociceptive properties and that an anxiolytic effect is mediated by benzodiazepnine receptors. In mammals, psychological stress is known to increase the core body temperature and this phenomenon is called stress-induced hyperthermia. We therefore examined the effect of YKS on cage-change stress induced hyperthermia (SIH) in mice. Male C57/BL mice weighting approximately 30-40 g were used in this study. Experiment 1: Mice were administered orally with YKS (100 or 1000 mg/kg) or vehicle 120 min before changing their home cages. Experiment 2: Mice were administered orally with diazepam (6 mg/kg) or vehicle 120 min before changing their home cages. Then, flumazenil (15 mg/kg) or vehicle was additionally injected subcutaneously 60 min before changing their home cages. Experiment 3: Mice were administered orally with YKS (1000 mg/kg) or vehicle 120 min before changing their home cages. Then, flumazenil (15 mg/kg) or vehicle was additionally injected subcutaneously 60 min before changing their home cages. We monitored core temperature by using telemetry. SIH of vehicle-treated mice were higher than that of both doses of YKS-treated mice and of diazepam-treated mice. Flumazenil reversed the diazepam-induced inhibitory effect on the SIH, but did not reverse YKS-induced inhibitory effect on the SIH. These results suggest that YKS reduces SIH via different mechanisms from benzodiazepines.

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Experimental evolution of pair bonding in the prairie vole (Microtus ochrogaster)

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The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent that unlike the majority of mammalian species, forms long-term relationships between sexual partners, or pair bonds. Other vole species such as the meadow vole (*M. pennsylvanicus*) do not form pair bonds and are relatively asocial. Comparative studies between vole species and within species studies of prairie voles have demonstrated that region-specific expression of the vasopressin 1a receptor (V1aR) in the male vole brain which appears to be driven by a polymorphic microsatellite element in the 5' regulatory region of the gene encoding V1aR is associated with both species differences and intraspecific variation in pair bonding behavior. When prairie voles were selectively bred based on the length of the microsatellite, males homozygous for the long microsatellite allele expressed higher levels of V1aR in the olfactory bulb and lateral septum compared to males with the short allele. In addition, males with the long allele expressed higher levels of paternal care, displayed higher levels of social interest, and were more likely to form partner preferences than males with the short allele. However, within prairie voles, it is still unclear if variation in V1aR expression is a major source of the heritable diversity in male pair bonding behavior. We hypothesized that male pair bonding behavior is a heritable trait and that through selective breeding, we could generate two lines of prairie voles where males display either a high propensity to form pair bonds or do not form pair bonds at all. If variation in the V1aR system is a major heritable variable leading to diversity in social bonding behavior, then we would predict that male prairie voles from the selected lines would also display different distributions of V1aR binding in the brain and that the length of the microsatellite element would differ between lines. After four generations of experimental evolution, we have already observed a trend towards divergence in behaviors between lines and are examining line differences in the location and density of V1aR in the brain as well as microsatellite length.

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Novel treatment for nephrogenic diabetes insipidus rat model using the sendai-virus vector carrying aquaporin 2 gene

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Nephrogenic diabetes insipidus (NDI) is a renal tubular disorder involving polyuria and polydipsia results from the unresponsiveness to vasopressin. The genetic defects in V2 receptor or the aquaporin2 (AQP2) results in the congenital form of the disease. The conventional medications including thiazide, water-restriction and prostagrandins are not effective enough to suppress polyurea symptoms. The lack of essential therapies makes the perioperative situations especially risky. Unconsciousness under anesthesia disables hydration by drinking, and to the polyuria disturbs the sodium balance on the transfusion therapy. In this study, we aimed to establish a fundamental technology for NDI treatment that can temporarily reverse the polyuria phenotype during the perioperative period. We generated a recombinant

Sendai virus vector (SeV) carrying a human AQP2 gene (AQP2/SeV). The therapeutic vector was administrated retrogradely via the urether in the lithium chloride (Li)-induced NDI rats completely depleting endogenous AQP2. Urine output and water intake decreased up to 40% for several days after the AQP2/SeV treatment. Immunohistological examination revealed the exogenous AQP2 expression in the collecting ducts. These results suggest the AQP2 gene transduced by AQP2-SeV was reoponsible for the water reabsorption in the renal medulla of NDI rats, resulting in urine concentration, decreased urine output, and water intake. This is the first report of successful gene therapy targeting the renal collecting ducts to restore the function of a deficient gene. This method is a new approach for correcting the fatal imbalance of water homeostasis in NDI patients, especially in the perioperative period.

P60

Green fluorescent vasopressin neurons express nuclear red fluorescence after osmotic stimulation in a double transgenic rat

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The up-regulation in the expression of mRNA or protein encoded by the c-fos gene is widely used as a marker of neuronal activation elicited by various kinds of stimuli. In order to facilitate the detection of activated neurons, we have generated transgenic rats expressing a fusion gene consisting of c-*fos* coding sequences in frame with monomeric red fluorescent protein 1 (mRFP1) under the control of c-*fos* gene regulatory sequences (c-*fos*-mRFP1 rats). Ninety minutes after intraperitoneal (i.p.) administration of hypertonic saline (9% [w/v] NaCl) in c-*fos*-mRFP1 transgenic rats, nuclear mRFP1 fluorescence was observed abundantly in brain regions known to be osmosensitive, namely the median preoptic nucleus (MnPO), the organum vasculosum lamina terminalis (OVLT), the supraoptic nucleus (SON), the paraventricular nucleus (PVN) and the subfornical organ (SO). Immunohistochemistry for Fos protein confirmed that the distribution of Fos-like immunoreactivity in non-transgenic rats was similar to those of mRFP1 fluorescence after i.p. administration of hypertonic saline in the transgenic rats. Several double transgenic rats were obtained from matings between transgenic rats expressing an arginine vasopressin (AVP)-enhanced green fluorescent protein (eGFP) fusion gene (AVP-eGFP rats) and c-*fos*-mRFP1 rats. In these double double transgenic rats, almost all eGFP neurons in the SON and the PVN expressed nuclear mRFP1 fluorescence 90 minutes after hypertonic saline administration. The c-*fos*-mRFP1 rats are a powerful tool which enable the facile identification of activated neurons in the nervous system.

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Gene therapy for nephrogenic diabetes insipidus using pseudotyped simian immunodeficiency virus-based lentiviral vector carrying aquaporin 2 gene

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Nephrogenic diabetes insipidus (NDI) is characterized by the inability of the kidney to concentrate urine in response to vasopressin. Congenital NDI results from mutations in the coding region for vasopressin-V2-receptor or aquaporin2 (AQP2) genes, and the conventional therapies are inefficient to rescue the polyuria phenotype in the patients. In the present study, we have illustrated recombinant Sendai virus vector (SeV) carrying a human AQP2 gene partially reversed polyurea symptoms in the lithium chloride (Li)-induced-NDI rat's when administered retrogradely from urether to the kidney. However, due to the nature of RNA virus-based vector, the therapeutic effects fade out within ten days after the administration.
In pursuit of the permanent therapy for congenital NDI, we switched the therapeutic vector to the pseudotyped simian immunodeficiency virus-based lentiviral vector (SIV). This vector is pseudotyped with vesicular stomatitis virus envelope glycoprotein G (VSV-G) to increase infection efficiency, and has utilized to treat various diseases including cystic fibrosis and pigmentary degeneration of the retina. In this study, we have confirmed VSV-G/SIV mediates eGFP gene expression in the mice kidney one month after the gene transfers. Next, we have generated VSV-G/SIV carrying AQP2 (AQP2/VSV-G/SIV). To evaluate the therapeutic effect, the vector was administered to two Li-induced NDI rats in a retrograde manner into the renal pelvis. During the observation period of over two weeks, either of the rats decreased water intake and urine output.
Our preliminary results suggest the possibility AQP2/VSV-G/SIV is applicable for the long-term therapy for NDI. However, further studies required in various aspects including safety test, improvement of the titer of the vector, optimization of the administration methods, and histological evaluations, before the clinical applications

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Mechanism of drinking induced by the sialogogue pilocarpine in rats

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Pilocarpine, a muscarinic receptor agonist, is a typical sialagogue to treat hyposalivation. Although peripherally applied pilocarpine makes the oral cavity wet by increasing saliva, it also induces water intake (1). When the thirst centers lying in the circumventricular organs and hypothalamus are activated, animals start to drink. We have reported that the subforni-

cal organ, a circumventricular organ, shows M3-receptor mRNA, including M2, M4 and M5, and M3-receptor immunoreactivity (2-3). Because of the absence of the blood-brain barrier in the circumventricular organs, peripherally administered drugs can more easily affect neurons in these regions directly. Thus, the peripherally administered pilocarpine may affect the thirst center in the brain and elicits the water intake. Mechanisms underlying the relationships between these events are unknown. Intracerebroventricularly injected pilocarpine induced water intake but not salivary secretion. Intracerebroventricularly applied atropine, a muscarinic receptor antagonist, suppressed the increased water intake by the intraperitonially and intracerebroventricularly applied pilocarpine. We tested which parts of brain were involved in these responses by using c-Fos immunohistochemistry. Intraperitonially injected pilocarpine increased the number of c-Fos immunopositive cells in some nuclei of the circumventricular organs, hypothalamus and medulla, which are related to thirst sensation. Intracerebroventricularly applied atropine suppressed the increased number of c-Fos immunopositive cells by the intraperitonially and intracerebroventricularly applied pilocarpine(4). We conclude that peripherally injected pilocarpine affects both the salivary glands and the thirst center in the central nervous system.

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P63

Differential regulation of the hypothalamic corticotropin-releasing hormone and the plasma corticosterone depending on stressors

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Response characteristics of the hypothalamic-pituitary-adrenal gland (HPA) axis have been reported to vary depending on stressors. Gamma-aminobutyric acid (GABA) neurotransnissions are implicated not only in the regulation of the HPA axis activity but also the brain reward pathway, and GABA contents in the brain regions increases or decreases in response to variety of stressors. Male Sprague-Dawley rats (300 ? 350 g) were exposed to restraint stress or cold stress for 2 h, respectively, and then transcardially perfused with 4 % PFA. Brain tissues were processed for mRNA *in situ* hybridization with cDNA probes of corticotropin-releasing hormone (CRH) or glutamic acid decarboxylase (GAD65), or for c-Fos immunohistochemistry in the hypothalamic paraventricular nucleus (PVN) and the nucleus accumbens (NAcb). Cardiac bloods were collected for plasma corticosterone assay. Either 2h of restraint or 2h of cold exposure markedly increased both the PVN c-Fos expression and the plasma corticosterone; however, the hypothalamic CRH expression was increased only by restraint, but not by cold, stress. Significant increases in GAD65 mRNA level and c-Fos expression in the NAcb were observed only by restraint stress, but not by cold exposure. Interestingly, GAD65 mRNA expression in the PVN was increased by cold stress, but not by restraint. These results suggest that the HPA activation responding to restraint stress comprises the hypothalamic CRH expression, and activation of other PVN neurons than CRH may be involved in the HPA activation by cold exposure. Additionally, it is suggested that differential expression of GAD65 may play roles not only in the HPA activation but also in the

brain reward system responding to stressors.

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Molecular configuration and neurosteroid sensitivity of tonic GABAA currents in supraoptic nucleus (SON) neurons

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Interactions between neurosteroids and GABA receptors have attracted particular attention in SON. Elaborate studies in SON have uncovered the fine molecular mechanism that PKC/phosphatase balance influenced the neurosteroid/$GABA_A$ receptors interaction. Although facilitation of the conventional phasic inhibition (IPSCs) has been considered the primary mechanism whereby neurosteroids influence neuronal excitability, $GABA_A$ receptors mediate a sustained tonic inhibition (I_{tonic}) as well as IPSCs in SON. Whether the steroid modulation on I_{tonic} is present in SON neurons is unknown. Here, we gained insights into the potential molecular configuration of $GABA_A$ receptors explaining neurosteroid sensitivity on I_{tonic} of SON neurons. A selective α_5 subunit inverse agonist L-655,708 blocked I_{tonic} in SON MNCs. L-655,708 caused outward shift in $I_{holding}$ (4.42±0.95, n=6), ranged ~40% of total I_{tonic} uncovered by additional BIC (20 μM). I_{tonic} enhanced by GABA (3 μM) was also blocked by L-655,708 in similar inhibition rate. A selective δ subunit agonist THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridin-3-ol, 1 μM) caused an inward shift in $I_{holding}$ (12.11±3.06 pA, n=10) without significant effect on IPSCs. Interestingly, THIP caused much smaller inward shift in $I_{holding}$ (7.54±2.27 pA, n=5, $p<0.05$) in the presence of L-655,708. Nevertheless, L-655,708 induced similar outward shift in $I_{holding}$ in the absence and presence of THIP, suggesting I_{tonic} enhanced by THIP is almost insensitive to L-655,708. Finally, submicromolar to micromolar concentrations of 3α,5α-THP and THDOC increased both I_{tonic} and the decay time of IPSCs in a concentration dependent manner. In summary, our results indicate that prognane steroids enhance I_{tonic} mediated by α5 and/or δ subunit containing $GABA_A$ receptors as well as IPSCs in SON.

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Aberrant intracellular calcium dynamics of neurohypophysis of thalidomide-induced autistic-like rats

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Autism is a psychological disease characterized by social impairment, communication deficits, and compulsive behavior [(1)]. Recent investigation suggested neuro-hormonal disorders (e.g., low level of oxytocin) may play a role in developing autism [(2)]. The present study aimed to assess whether a dysfunction of the intracellular calcium ($[Ca^{2+}]i$) signaling mechanism oc-

curs in autistic-like conditions. To this end, we analysed $[Ca^{2+}]i$ dynamics in thalidomide-induced autistic-like rat neurohypophyses. Slice specimens of male rat neurohypophyses were loaded with Indo-1 (a Ca^{2+} indicator) and spatio-temporal $[Ca^{2+}]i$ dynamics during membrane depolarization were examined by real-time confocal microscopy. When the control pituitary was stimulated for 1 min by high K^+ (50 mM), $[Ca^{2+}]i$ of both nerve endings and pituitocytes increased, especially the $[Ca^{2+}]i$ dynamics of nerve endings were prominent. The increase of nerve endings was rapid and then declined gradually. The plateau of $[Ca^{2+}]i$ increase was caused by Ca^{2+} replenishment from the external environment. In thalidomide-treated rats, the neurohypophysis showed a slower and weaker increase, and faster decrease. Experiments in extracellular Ca^{2+} free conditions, Gd^{3+} or thapsigargin treatment indicate the abnormality of voltage-operated Ca^{2+} channel (VOC) and capacitative Ca^{2+} entry (CCE) in thalidomide-treated autistic-like rats. Dysfunctions of VOC and CCE of neurohormonal elements may play a pivotal role in drug-induced autistic-like conditions.
In addition, we analysed whether a behavioral dysfunction occurs in thalidomide-induced autistic-like rats by the multiple T-maze test.

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Alteration of gene expression of hypophysial hormones and their receptors in environmental chemical-caused hyperactive rats.

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The very large numbers of environmental chemicals that now exist have made assessing their neural risks challenging. Recent evidence points to an important effect of exposure to environmental neurotoxicant chemicals on the marked increase seen in neurodevelopmental disorders. We have demonstrated that exposure to some environmental chemicals, such as bisphenol A, octylphenol, nonylphenol, dibutylphthalate (DBP), diethylhexylphthalate (DEHP), and concluded that environmental chemicals seem to be neurotoxic to the developing rat brain, but it is still unclear whether their effects on the developing brain result from their endocrine disrupting activity or some other as yet uncharacterized process.
Therefore, we carried out DNA microarray to investigate cellular effects of the chemicals. Total RNA was isolated from rat brains at 8 weeks of age and was then transcribed to cDNA. The DNA macroarray was performed using Rat 1.2 array membranes (BD Biosciences Clontech), which had 1,176 cDNA, including pituitary and hypothalamus hormones and their receptors.
It was found that some environmental chemicals altered the gene expression of hypophysial hormones and their receptors. Nonylphenol decreased the levels of gene expression of FSH beta, TSH beta, TSH receptor, and vasopressin receptors, whereas it increased those of GH receptors. Octylphenol and DCHP limitedly decreased the levels of vasopressin receptors, 0.73 and 0.59 fold, respectively as far as tested. It was particularly notable that gene expression of dopamine transporter was largely decreased by all chemicals tested (i.e. bisphenol A, nonylpheol, octylphenol, DBP, DCHP, DEHP).
Thus, environmental chemicals caused hyperactivity in the rat, probably regulating the levels of gene expression of both hypophysial hormonal systems and dopaminergic system.

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Region-specific changes in stimulated monoamine release in the supraoptic nucleus of pre-parturient rats

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To investigate changes in excitatory drive which may be involved in the activation of oxytocin neurons in the supraoptic nucleus (SON) at parturition, concentrations of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) were measured with high pressure liquid chromatography in sequential 10min microdialysis samples taken from dorsal or ventral SON regions of urethane-anaesthetised rats. The basal release, and effects of cholecystokinin (CCK, 20?g/kg *i.v.*) and antidromic activation of magnocellular neuron axons via the neural stalk (50Hz stalk stimulation, 3s every 10s for 30min) were compared in age-matched virgin female and 22 day pregnant rats. Basal NA release from dorsal and ventral SONs was increased in day 22 pregnant (dorsal: 301±9; ventral: 802±45pg/ml; n=12) compared to virgin rats (dorsal: 146±6; ventral: 360±15pg/ml, n=12, $P<0.05$). In the dorsal SON in virgins, NA release increased after either CCK (100% increase) or 50Hz stalk stimulation (120% increase) and evoked release was greater in pregnant rats (240% and 200%, increase respectively, $P<0.05$). In the ventral SON, CCK inhibited NA release (virgin: 64% and pregnant: 87% decreases) while 50Hz stalk stimulation increased NA release (virgin: 70%; pregnant: 69% increase). In rats given repeated periods of 50Hz stalk stimulation the second stimulation period (60 min later) increased NA release in both dorsal (virgin 234%; pregnant 390% increases) and ventral SON (virgin: 60%; pregnant: 75% increases). However, in the dorsal SON of both pregnant and virgin rats, the response to the second stimulus was significantly larger than that to the first stimulation period, suggesting facilitation of NA release only in the dorsal region of the SON. Changes in 5-HT were similar to NA, but dopamine levels were not changed under any experimental conditions. Confirming previous results showing increased basal NA and 5-HT release in late pregnancy, our results also suggest that there is an SON region-specific increase in responsiveness of NA and 5-HT release. As the majority of oxytocin neurons reside in the dorsal SON and NA is released in the SON during parturition we are currently investigating the role of centrally-released oxytocin in facilitating NA and 5-HT release in the SON perinatally.

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Identification of transcripts regulated in response to dehydration in mouse magnocellular neurons of the hypothalamic-neurohypophyseal system (HNS).

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Maintaining osmotic stability is critical for life: instrumental to this response is the hypothalamic neuropeptide arginine vasopressin (Avp). Following an osmotic stimulus such as dehydration, circulating levels of Avp increase and in its classical role as an antidiuretic hormone, Avp promotes renal collecting duct permeability and thus water conservation. Release of Avp during dehydration is also accompanied by extensive remodelling of SON and PVN neurons in terms of morphology, electrical properties and secretory activity. These changes

promote synthesis and delivery of Avp in order to restore fluid balance. We have used microarray and *in situ* hybridisation (ISH) techniques to identify regulated transcriptome targets in response to dehydration in mice. Microarray analysis (Affymetrix) was performed on total SON RNA extracted by laser-capture microscopy from mice subjected to either 48h dehydration or controls. From this analysis four transcripts that expressed robust up-regulation were identified for further validation by ISH. Cebp beta, Atf4, Creb3l1 and prodynorphin mRNA showed significant ($P<0.05$) increases in expression in the SON and PVN of 48h-dehydrated mice in comparison to controls. Moreover previous microarray and ISH analysis also highlighted Cebp beta, Atf4 and Creb3l1 to be regulated in response to dehydration in rats, (1) whilst prodynorphin is colocalised and up-regulated in parallel with vasopressin mRNA expression in rat magnocellular neurons of the hypothalamus, subjected to osmotic challenge. Interestingly, comparisons between mouse and rat chronic dehydration microrarray and ISH data have also highlighted differences in transcription regulation between the two species. Most notably was the absence in regulation in Caprin 2 and Tnfaip6 transcripts in chronically dehydrated mice when compared to a rat dehydration paradigm in both microarray and ISH analyses. These data therefore identifies both conserved and differentially regulated HNS genes between mouse and rat in response to an osmotic challenge. Furthermore, conserved regulatory genes may function as important mediators in physiological responses to dehydration and therefore are prime candidates for further study.

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P69

Expression of vasopressin and vasopressin V1a and V1b receptors in the rat anterior olfactory nucleus

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Utilizing a transgenic rat that expresses an eGFP-vasopressin fusion gene (1), we detected previously undescribed populations of vasopressin neurones in the main (MOB) and accessory olfactory bulbs (AOB). Here we describe another population of vasopressin expressing neurones within the anterior olfactory nucleus (AON). In contrast to the large neurones in the MOB and AOB, the cells in the AON are smaller with less extensive dendritic projections and in are found in banded groups, in the *pars externa* and in the outer plexiform layer of the AON. To determine the neurochemical composition of the vasopressin expressing cells we used fluorescent immunohistochemistry to label GFP or vasopressin and in combination with specific antibodies against the neurotransmitter, GABA and glutamate, and the calcium sensing proteins, calretinin and calbindin-D-28K. In addition, we examined whether AON vasopressin expressing cells are also express vasopressin V1a and/or V1b receptors. Our data show that the vasopressin cells in the AON are immuno-positive for GABA and calbindin-D-28K and express both the V1a and V1b receptors receptor subtype suggesting vasopressin may regulate output from this region to higher brain centres at the level of the AON. The physiological relevance of this finding requires further studies. Using immediate early gene expression, we have shown modulation of vasopressin cell activity in the AON in response to various social stimuli(2). The AON is a central olfactory cortical structure with extensive connections to the olfactory bulb, and to the rest of the brain through the piriform cortex, suggesting that olfactory vasopressin can modulate information processing at the level of the AON.

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P70

Calcineurin/NFAT Pathway may be a regulator of the mice parturition

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[Problem]The oxytocin (OT)?oxytocin receptor (OTR) system plays an important role in mammalian parturition. However, we found both OT-dificined mice and OTR-deficient (OTRKO) mice are fertile and deliver at term without birth defects, thus alternative pathways inducing parturition can be hypothesized.
[Methods] We tested the gene expression profile of OTRKO mice and wild type (WT) mice using suppressive subtractive hybridization, and focused on the calcineurin ? nuclear factor of activated T cells (NFAT) pathway, which is one of the calcium dependent signalling pathway. We examined the expression and localization of this pathway in mouse parturition.
[Results] Calcineurin A and NFATc1 were detected in the decidua of pregnant uteri at term using immunohistochemistry (IHC) in both WT and OTRKO mice and the NFATc1 was shown not only in the cytoplasm but also in the nucleus, which suggests the activity of this pathway in mice uterus at term. We identified increased calcineurin A and NFATc1 mRNA levels during pregnancy. Moreover, injection of FK506, the inhibitor of this pathway, prolonged the delivery of the first pup.
[Conclusion] Our findings suggested that the calcineurin ?NFAT pathway might play a substantial role in initiation of labor.

P71

Genome-wide identification of CREB target genes in oxytocin and opioid sensitive human neuroblastoma cells

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Oxytocin (OT) and Arginine vasopressin (AVP) have a regulatory role in opioid analgesia and subsequent development of analgesic tolerance through direct activation of central oxytocin and/or vasopressin receptors. To delineate roles of posterior pituitary hormones in the development process of tolerance to opioid analgesia, we established cellular model of morphine-induced adenylate cyclase super-activation, a biochemical hallmark of morphine tolerance, in oxytocin-sensitive human neuroblastoma cells and analyzed cellular adaptations to chronic

morphine treatment. Pre-incubation of oxytocin with morphine significantly accelerated adenylate cyclase activity in this cell line. Cyclic AMP response element-binding protein, CREB, is a key transcription factor in the development of opioid tolerance, however unbiased view of CREB binding sites throughout the entire human genome and their relationship to nearby transcripts in opioid-sensitive cell has not been fully elucidated. We report here our results on the chromatin-immunoprecipitation (ChIP) assay with anti-CREB antibody and whole genome tiling array analysis applied to morphine and oxytocin-treated or na?ve SK-N-SH human neuroblastoma cells, which express native m-opioid receptors. In a total of 1913 CREB-associated genomic fragments, a large fraction (85.2%) of the DNA segments were localized to upstream promoter or within gene loci, while the rest of segments were localized in intergenic regions. Between two distinct cell types, SK-N-SH and human embryonic kidney cells, the CREB-DNA associations were conserved and ChIP-enriched segments overlapped at 128 regions (6.7 %). The conserved CREB-binding sites include promoters of known CREB-regulated genes, such as c-FOS, FOSB, cAMP responsive element modulator, and calmodulin 2, and multiple new CREB target genes. Morphine treatment did not change CREB binding in a majority of promoters. Our analysis thus identified novel molecular signatures for opioid tolerance, and critical contribution of the posterior pituitary hormone to opioid analgesia.

P72

Induction of the Arginine Vasopressin-enhanced Green Fluorescent Protein Fusion Gene in the Rat Locus Coeruleus

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We examined the effects of i.c.v. administration of colchicine on the expression of arginine vasopressin (AVP)-enhanced green fluorescent protein (eGFP) fusion gene in the hypothalamic and extra-hypothalamic areas of these transgenic rats. In the vehicle i.c.v. administered rats (control) eGFP fluorescence was observed in the the supraoptic nucleus (SON), the magnocellular division of the paraventricular nucleus (PVN), the suprachiasmatic nucleus (SCN), the median eminence and the posterior pituitary (PP). Two days after i.c.v. administration of colchicine eGFP fluorescence was markedly increased in the SON, the magnocellular and parvocellular divisions of the PVN, the SCN, the median eminence and the locus coeruleus. In the PP, the intensity of the eGFP fluorescence was similar in both control and cholchicine treated animals. No eGFP fluorescence was observed in the parvocellular division of the PVN and in the locus coeruleus of control rats. Furthermore, in both colchicine treated or untreated rats, no eGFP fluorescence was observed in other noradrenergic cells, such as those located in the nucleus tractus solitarii, the ventrolateral medulla and the adrenal medulla. Immunohistochemical staining for eGFP confirmed the distribution of fluores-

cence in both groups. In colchicine administered groups, immunohistochemistry for tyrosine hydroxylase (TH) revealed that the eGFP fluorescence was co-localised with TH-immunoreactivity in the locus coeruleus. Similarly, *in situ* hybridisation histochemistry for the eGFP mRNA revealed that a significant increase in the gene expression in the locus coeruleus as well as in the SON and the PVN 12-48 hours after administration of colchicine. Our results indicate that the synthesis of AVP-eGFP is upregulated in noradrenergic neurones in the locus coeruleus after colchicine administration. This implies that AVP and noradrenaline, originating from locus coeruleus neurones may play a role in response to stress.

P73

Stress-induced behavior and plasma oxytocin levels during development are regulated dually by breast milk and hypothalamic ADP-ribosyl cyclase of CD38 in mice

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Oxytocin (OT), a neurohormone involved in reproduction, also plays a critical role in social behavior from rodents to humans and is related to autism. CD38, a transmembrane protein, is highly expressed in the hypothalamus (1). Neuronal roles of CD38 have been recently revealed (2). CD38-dependent regulation of OT secretion is critical for social behavior in adult mice. But it has not been examined in infants or during development. To assess the above question, we used separation from the mouse dam in 7-day old pups. During such isolation stress, locomotor activity was higher in CD38 knockout ($CD38^{-/-}$) pups than in wild-type controls ($CD38^{+/+}$). The frequency of ultrasonic vocalization (USV) was lower in $CD38^{-/-}$ pups than in $CD38^{+/+}$ pups (3). However, the difference between the two genotypes seems to be less severe than those in OT knockout or OT receptor knockout mice. To explain this, we measured plasma OT levels. The OT level was not lower in $CD38^{-/-}$ pups during the period from 1?3 weeks after birth, but was significantly reduced after weaning (?3 weeks), as reported before (1). ADP-ribosyl cyclase activities in the hypothalamus and pituitary were markedly lower from 1 week after birth in $CD38^{-/-}$ mice and were consistently lower thereafter to the adult stage (2 months old). In addition, we found that the mammary gland and breast milk of lactating dams were rich in OT. These results demonstrate that the reduced severity of behavioral abnormalities in $CD38^{-/-}$ pups is due to high levels of plasma OT taken from the dam's milk. These results suggest that CD38-dependent secretion of OT into the brain from hypothalamic neurons is important for social behavior and that OT levels are dually regulated before the critical switching time (at weaning 3 months after birth) to assist in social brain development.

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P74

Centrally administered adrenomedullin 5 activates oxytocin-secreting neurons in the hypothalamus and elevates plasma oxytocin level in rats

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Adrenomedullin (AM), which was discovered from the human pheochromocytoma, is a multifunctional peptide that belongs to the calcitonin gene-related peptide (CGRP) family that is composed of CGRP, AM and amylin. In teleost fish, AM peptides were identified as five AMs (AM1-5), and they form an independent subfamily. Takei *et al*. searched the orthologs of the AMs in the genome and established sequence tag (EST) databases and identified AM2 and AM5 genes in mammals (1, 2). In this study, we examined the effects of intracerebroventricular (icv) administration of the AM5 in conscious rats. AM5 (2 nmol/rat) increased Fos-like immunoreactivity (LI) at 90 min post-injection in the hypothalamic and brainstem regions. The activation was particularly eminent in the supraoptic nuclei (SON) and the paraventricular nuclei (PVN). Dual immunostaining for Fos/oxytocin (OXT) and Fos/arginine vasopressin (AVP) revealed that OXT-LI neurons predominantly colocalized Fos-LI compared with AVP-LI neurons in both the SON and PVN. Consistently, plasma OXT levels significantly increased 5 min after icv administration of AM5 (1 nmol/rat) and remained elevation for 30 min, while plasma AVP levels did not change for 30 min after injection. *In situ* hybridization histochemistry showed that AM5 (0.2, 1 and 2 nmol/rat) caused marked induction of the c-*fos* gene expression in the SON and PVN. This induction was partially reduced by pretreatment with both calcitonin gene-related peptide (CGRP) receptor antagonist CGRP-(8-37) (3 nmol/rat) and AM receptor antagonist AM-(22-52) (27 nmol/rat). These results suggest that centrally administered AM5 activates OXT-secreting neurons in the hypothalamus partly through the known AM/CGRP receptors and elicits secretion of OXT into the systemic circulation in rats.

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P75

Influence of hormone treatment and mating conditions on anxiety-related behaviour and central oxytocin release in female rats

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Close social interactions, like sexual activity and partner intimacy, have positive health consequences including attenuated stress responses and anxiolysis, as shown in men and male rats (1, 2). Positive consequences were also reported for women in the context of emotional

stress (1). Here, we tested the hypothesis that both steroid priming as well as mating alter state anxiety in female rats and that the latter effect is dependent on whether the engagement in sexual activity occurs voluntarily.
Virgin female rats were ovariectomized, some rats were primed (estradiol 200 µg / 0.2 ml oil; progesterone 500 µg / 0.2 ml oil) and either single-housed (non-mated) or exposed to a male rat for 30 min (mating group). Mating was performed under unpaced (inescapable) or paced (the female could escape into a neighbouring compartment) mating conditions. Thirty min after mating, female rats were tested for anxiety-related behaviour in either the black-white box (BWB) or on the elevated plus-maze (EPM).
Priming significantly reduced state anxiety as indicated by an increased exploration of the lit compartment of the BWB and the open arms of the EPM. Interestingly, mating under unpaced conditions increased the level of anxiety. In contrast, in paced mated females, state anxiety remained at the low level found in primed females.
Central release of the neuropeptide oxytocin (OXT) has been implicated in female sexual and emotional behaviours and it was found to mediate the anxiolytic effect of sexual activity in male rats (2). Using microdialysis during ongoing behavioural testing and mating, we could reveal that OXT is released within the brain, specifically within the hypothalamic PVN, during paced, but not unpaced mating. No differences in hypothalamic OXT release were found between primed and non-primed females indicating local OXT release being independent of the acute steroid treatment.
The results provide evidence that an anxiolytic effect of hormonal priming is necessary to promote the close proximity to the larger male and mating. They further show negative emotional consequences of unpaced mating, whereas after voluntary paced mating the low level of anxiety remains, which is likely due to an activation of the hypothalamic OXT system. Supported by DFG and BMBF.

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P76

TRPV1 gene deficiency attenuates miniature EPSCs potentiation induced by mannitol and angiotensin II in supraoptic magnocellular neurons

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The release of arginine vasopressin (AVP) from the magnocellular neurosecretory cells (MNCs) in the supraoptic nucleus (SON) is crucial for body fluid homeostasis. The neuronal activity of the MNCs is modulated by synaptic inputs and humoral factors. A recent study demonstrated that an N-terminal splice variant of the transient receptor potential vanilloid type 1 (N-truncated form of the TRPV1) is essential for osmosensory transduction in the SON. In the present study, we examined the effects of mannitol and angiotensin II on the miniature excitatory postsynaptic currents (mEPSCs) in the SON MNCs by using whole-cell patch-clamp recording in *in vitro* slice preparation. Mannitol (60 mM) and angiotensin II (0.1 mM) increased the frequency of mEPSCs significantly without affecting the amplitude. These effects were attenuated by pre-exposure to a non-specific TRPV channel blocker, ruthenium red (10 mM). The potentiation of mEPSCs by mannitol was not mimicked by a TRPV1 ago-

nist, capsaicin, and also not attenuated by selective TRPV1 blockers, capsazepine. Protein kinase C (PKC) was involved in angiotensin II-induced potentiation of mEPSCs. The effects of mannitol and angiotensin II on the SON MNCs in *trpv1* knockout mice were significantly attenuated as compared with those in wild-type mice counterparts. These results suggest that hyperosmotic stimulation and angiotensin II modulate mEPSCs through capsaicin-insensitive TRPV1 channels in the presynaptic terminals of the SON.

P77

The MAPK signalling pathway within the PVN is critically involved in both lactation- and oxytocin-induced anxiolysis

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Interest in the neuropeptide oxytocin (OXT) has grown substantially in recent years, which has been precipitated by the discovery of a multitude of behavioural and neurochemical effects, both in animal and human studies. Of particular relevance, we could previously demonstrate that intra-PVN administration of OXT resulted in anxiolysis in male rats, mediated *via* activation of the MAPK pathway. In the present study we wanted to extend these findings to female rats and in particular to lactating dams. The time after birth is characterised by attenuated stress responsivity and anxiolysis in the dam, accompanied by activation of the brain oxytocinergic system. Therefore, we hypothesised that the MAPK signalling pathway would be up-regulated in lactation and that this contributes to the well-documented anxiolysis observed during this period. Indeed, we could demonstrate under basal conditions that pERK levels were significantly higher in hypothalami from lactating dams compared with virgins. Moreover, both the lactation-associated anxiolysis and increased pERK expression were reversed by administration of the MEK inhibitor, U0126. In contrast, in virgin females, U0126 administration did not affect either parameter. As observed in male rats, OXT administration was shown to increase pERK expression in the hypothalami of virgin female rats, as well as resulting in anxiolysis. Both of these effects were prevented by pre-administration of U0126. In contrast, OXT infusion did not affect either parameter in lactating rats. Taken together these results suggest that elevated pERK levels within the hypothalamus mediate the anxiolysis observed following OXT administration and in lactation.

P78

Identification of a new hOTR/Gai 'functionally selective' peptide using a BRET assay.

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The human oxytocin receptor (OTR) is a promiscuous receptor functionally coupled to various G-proteins (Gaq-11, Gai and Gah). The multiple receptor coupling is particularly relevant because the activation of OTRs coupled to different G protein pathways can also trigger opposite cellular response, e.g. OTR coupling to G(i) inhibits, whereas its coupling to G(q) stimulates, cell proliferation (1). Moreover, specific ligands may possess different intrinsic efficacy on the different signaling pathway, a phenomenon referred to as “agonist-directed trafficking of receptor stimulus”, “biased agonism” or “functional selectivity”. The existence of these analogues is consistent with a multistate model of receptor activation in which ligands can induce specific receptor conformations capable of differentially promoting the coupling of a single receptor to different G-proteins.
To better define the signaling pathways activated by the OTR, we performed a BRET assay measuring G protein activation, where the energy transfer occurs between the human G protein alpha subunits (Gaq and Gai1, Gai2, Gai3) fused to Renilla Luciferase and the gamma subunit (Gg) of the hetherotrimeric G-proteins fused to GFP10.
Starting from our knowledge from atosiban (1), the first OT derivative that acts as a competitive antagonist on OTR/Gq coupling and as an antagonist on OTR/Gi coupling, we used a rational approach for the design of other OTR ‘functional selective ligands’ and we investigated a series of OT analogs as well as four peptidic OTR/Gq antagonists. These analogs were then screened by BRET for their coupling selectivity. The results obtained led us to the identification of a new OTR/Gi “functional selective peptide” that has a 1000 times greater affinity for hOTR than atosiban, a feature that makes this analog a promising candidate as new pharmacological tool.

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P79

The function-related plasticity of the hypothalamo-neurohypopyseal system is governed by global transcriptional events

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The hypothalamo-neurohypopyseal system is a highly specialised brain axis that is primarily responsible for the discharge of the closely related hormones vasopressin and oxytocin into the circulation. In response to osmotic stimulus such as dehydration, cardiovascular stimulus such as hemorrhage, or reproductive events such as parturition and lactation, neurons of the supraoptic (SON) and paraventricular (PVN) nucleus undergo a myriad of morphological, biosynthetic and electrical changes to facilitate the appropriate release of these hormones. We hypothesise that transcriptional events underpin not only this plasticity but also the resultant hormone release and have accordingly used a whole genome expression approach to investigate this premise. Affymetrix microarrays that represent complete rat transcriptome coverage have been interrogated using tissue from the SON, PVN and the neurointermediate lobe (NIL) taken from either euhydrated or 72-hours dehydrated male Sprague Dawley rats. In addition we have investigated the transcriptional changes that occur in the female SON following both dehydration and 11-days lactation. In the SON, some 183 genes are differentially by greater than 2-fold in the male SON and 99 genes in the female SON following 72-hours dehydration, while lactation resulted in 433 regulated genes with satisfactory overlap between the three datasets. The same cut-offs result in fewer genes regulated by dehydration

in the PVN, just 12, presumably a function of the more heterogeneous population of neurons in this structure. Comparison between the SON and PVN also reveals a degree of overlap between these tissues. The NIL, resulted in 254 genes, of which 26 are regulated in different directions by dehydration when a 1.5-fold cutoff is applied. Lastly, a rational mathematical approach has been taken to describe the underlying gene networks that describe the plasticity noticed in the SON.

P80

Prenatal immune stress attenuates juvenile social play behavior and vasopressin mRNA expression in the amygdala in male but not in female rats

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Prenatal immune stress is a risk factor for behavioral disorders such as schizophrenia and autism, suggesting that immune stress may interfere with the development of the neural circuitry driving social behavior. This study explored the behavioral and physiological consequences of prenatal immune stress in rats. Lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, or physiological saline was administered to pregnant rats on embryonic day 15. Play behavior of pups was video-taped from 22-45 days of age. Animals were tested in the home cage with all littermates present, or in age- and sex-matched pairs after one hour of isolation. Males showed more play behavior than females in any setting at all ages. LPS tended to reduce play behavior in the home cage, but differences did not reach significance ($p=0.06$). However, LPS significantly decreased the frequency of play in male offspring in treatment-matched pairs at all ages tested. Although there was no difference in the frequency of play behaviors between LPS- and saline-treated rats in mixed treatment pairs, LPS-treated rats initiated play significantly less often than saline-treated rats. This suggests that LPS may not interfere with the ability to respond to social play but may alter the tendency to initiate play. LPS did not significantly influence the frequency of play behaviors in female rats. LPS also reduced vasopressin mRNA expression in the medial amygdala of males but not of females. As preliminary results from our lab suggest that intracerebroventricular injections of aV_{1a} antagonist blocks play behavior in rats, the reduction in vasopressin mRNA may underlie the reduction in social play. The stronger effects of LPS in males than in females may help understand the etiology of behavioral disorders that affect males more than females, such as autism.

P81

a1A adrenergic receptors are not required for sustained stimulation of vasopressin release by ATP and phenylephrine

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Co-exposure of hypothalamo-neurohypophyseal system (HNS) explants to ATP and phe-

nylephrine [PE; an a1-adrenergic receptor (a1-R) agonist] evokes a synergistic stimulation of vasopressin (VP) release that is sustained for hours (2). Recruitment of the non-desensitizing P2X2/3 and P2X7 subtypes of ionotropic purinergic receptors is required for this response (1). To investigate the role of PE in inducing this recruitment, we initiated studies to determine the type of a1-R required for the synergistic response. Three a1-R subtypes are expressed in supraoptic neurons (SON): a1A-R, a1B-R, and a1D-R. These subtypes differ in protein binding partners and signaling cascades resulting in differences in their rate of agonist induced internalization, agonist-independent cell localization, and effects on gene expression. As reported at this meeting (Song and Sladek), specific a1A-R antagonists blocked the increase in $[Ca^{++}]i$ induced by PE in SON. We investigated the ability of the same a1A-R antagonist to prevent or interrupt the sustained stimulation of VP release from HNS explants. WB4101(100 μM) was added to the perifusion medium either 1.5 hours in advance of ATP+PE (both at 100μM) or 2 hrs after the sustained response to ATP+PE had been established. Early addition of WB4101 did not significantly alter basal VP release compared to time control explants. It also did not prevent sustained stimulation of VP release by ATP+PE. Furthermore, although continued exposure to PE is required to maintain the elevated VP release induced by ATP+PE, addition of WB4101 2 hrs after ATP+PE did not disrupt the sustained stimulation of VP release. These findings demonstrate that additional a1-R subtypes are required for the sustained VP response to ATP+PE. Since sustained stimulation of VP release is required to maintain cardiovascular function during dehydration, hemorrhage, and sepsis, identifying the cellular mechanisms underlying this response is clinically significant. Supported by NIH RO1-NS027975 and AHA GIA #0855728G.

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P82

Maternal separation impairs social recognition due to a lack of septal vasopressin responsiveness in adult male rats

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Social cognition deficits are central to neurodevelopmental disorders like autism and schizophrenia and were associated with impaired brain vasopressin (VP) functions. We used maternal separation (MS, daily, day 1-14) to investigate the role of developmental stress on social recognition abilities and underlying mechanisms in juvenile (5 weeks of age) and adult (16 weeks of age) male Wistar rats. Social discrimination was defined by increased investigation of a novel 3-week-old juvenile compared with a previously encountered (same) juvenile by the experimental rat. At juvenile age, both control and MS rats demonstrated social discrimination abilities at inter-exposure intervals of 30 and 60 min. By contrast, in adulthood MS rats failed to discriminate between the same and novel juvenile after an inter-exposure interval of 60 min. The social memory deficit of adult MS rats was accompanied by a lack of an increase in septal VP release, as found in control rats, during social memory acquisition. This lack of local rise in VP in adult MS rats was social stimulus-specific as exposure to forced swimming stimulated local septal VP release in both control and MS rats. Local application of synthetic VP into the septum via reversed microdialysis during social memory acquisition improved social discrimination in adult MS rats after an inter-exposure interval of 60 min. The observed

deficits in social recognition and septal VP responsiveness indicate MS-induced alterations in the processing of social cues in brain regions upstream from the septum. Alterations in social cue processing may result in additional, inappropriate social behaviours like enhanced aggression as shown previously.
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Systemic secretin increases the electrical activity of supraoptic nucleus (SON) OT neurones and stimulates oxytocin (OT) secretion in the rat

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Secretin, a 27 amino acid brain-gut peptide from duodenal S-cells, increases Fos expression in the SON upon systemic administration (40µg/kg; i.v) [1]. Secretin receptors are expressed in the SON [2] and central administration of secretin (0.1-10µg) increases plasma OT, while *in vitro* secretin increases dendritic OT release from the isolated SON [3]. We hypothesized that physiological systemic doses of secretin, simulating postprandial release, would excite magnocellular OT neurones. Hence, we examined the effect of secretin given intravenously (?1?g/rat; i.v.) [4] on the electrical activity of SON OT neurones and OT secretion in urethane anaesthetized female rats.
Secretin dose-dependently (0.01, 0.1 and 1µg; i.v.) increased SON OT neurone firing rate ($P<0.001$; 1-way ANOVA). The mean +/- s.e.m. basal firing rates of 3.5 ± 0.62, 4.06 ± 0.4 and 6.05 ± 0.91 spikes/s, respectively, of OT neurones in rats given 0.01µg (n=4), 0.1µg (n=26) and 1µg (n=3) secretin, was increased by 0.65 ± 0.34, 1.74 ± 0.16 and 4.17 ± 1.4 spikes/s, respectively, within 2 min after injection (paired t-test: pre vs post-secretin: 0.01?g: $P=0.109$; 0.1µg: $P<0.001$; 1µg: $P=0.04$). Secretin was more effective than cholecystokinin (CCK): the excitatory response to low-dose secretin (0.1µg i.v.; n=26) was greater than to a higher CCK dose (25µg/kg i.v.; n=45), $P<0.001$(Kruskal-Wallis 1-way ANOVA].
Plasma OT concentration was also increased dose-dependently by secretin (0.1 and 1µg; i.v) (n=6). The basal OT concentration of 42.7 ± 5.38pg/ml was elevated to 103.1 ± 23.72pg/ml ($P=0.031$; 1-way RM ANOVA) and 172.7 ± 25.41pg/ml ($P=0.001$; 1-way RM ANOVA) 5min after 0.1µg and 1µg secretin, respectively (0.1µg vs 1µg: $P<0.001$; 1-way RM ANOVA).
As both systemic secretin and OT are involved in regulating gastrointestinal functions and natriuresis, systemically released secretin might act partly through OT. The effect of physiological doses of systemic secretin on central OT release is under study.

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Hyponatremia-Induced Osteoporosis

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There is a high prevalence of chronic hyponatremia (HN) in the elderly, frequently due to inappropriate secretion of antidiuretic hormone (SIADH). Recent reports have shown adverse effects of HN on gait stability leading to an increased risk of falls, a risk factor for fractures. We tested the hypothesis that prolonged HN also contributes metabolically to bone loss by activating bone resorption to release stored sodium from bone. Using a rat model of SIADH, HN was maintained for 3 months in 22 month-old male F344 Brown Norway hybrid rats (F344BN) by infusing desmopressin (5 ng/h) and feeding a liquid diet. Normonatremic control aged F344BN rats also received desmopressin, but were pair-fed the same diet in solid form. Biweekly measurements of bone mineral density (BMD) by DXA demonstrated that HN induced greater progressive bone loss than aging alone (AP spine -17% vs -9%; total femur -11% vs -6%; proximal tibia -12% vs -3%; $p<0.05$). In 12 month-old male F344BN rats, HN for 3 months caused severe trabecular and cortical bone losses by micro-computed tomography. Histomorphometry and in vitro osteoclastogenesis studies indicated that the bone loss was primarily due to increased bone resorption. Analysis of the Third National Health and Nutrition Examination Survey via linear regression demonstrated that HN was independently associated with increased odds of osteoporosis (T-scores$\geq$-2.5) at the hip in humans (odds ratio=2.85; 95% CI 1.03-7.86, $p<0.01$) after adjustment for age, sex, race, BMI, physical activity, serum 25(OH)D levels, and history of diuretic use and smoking. Our results represent the first demonstration that chronic HN causes substantial bone mineral loss, a metabolic effect increasing fracture risk. Cross-sectional data in humans showing that HN is associated with significantly increased odds of osteoporosis are consistent with the experimental data in rats, and suggest that bone quality should be assessed in all patients with chronic HN (supported by AG029477).

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